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LINOLEIC ACID UPTAKE BY BRUSH BORDER MEMBRANE VESICLES FROM ALONG THE CRYPT-VILLUS AXIS OF RABBIT JEJUNUM

BY

Robert J. Fingerote Ⓒ

A THESIS

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Robert Fingerote

ROBERT J. FINGEROTE
152 GLENGARRY AVENUE
TORONTO, ONTARIO
M5M 1E2

DATE: June 23, 1992

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **"LINOLEIC ACID UPTAKE BY BRUSH BORDER MEMBRANE VESICLES FROM ALONG THE CRYPT-VILLUS AXIS OF RABBIT JEJUNUM"** submitted by **Robert J. Fingerote** in partial fulfillment of the requirements for the degree of **Master of Science** in **Experimental Medicine**.

A.B.R. Thomson, MD, PhD (supervisor)

C.I. Cheeseman, PhD

R Fedorak

R.N. Fedorak, MD

Michael I. McBurney

M.I. McBurney, PhD

Date: Jan 7/90

DEDICATION

There is a special happiness that comes with achieving successes, such as the happiness that comes with achieving a Master of Science degree. However, with every happiness there is a sense of sorrow that people we hold dear are not with us to share in this happiness.

I dedicate this thesis to the memory of my parents, Sharon and Sam Fingerote.

ABSTRACT

Glucose uptake by the jejunal brush border membrane (BBM) varies along the crypt-villus axis (CVA). Does uptake of the essential long chained fatty acid linoleic acid (LA) also vary along this axis? Using agitation techniques, five enterocyte fractions were sequentially isolated from female New Zealand white rabbit jejunum. A sixth and final fraction of lower-villus/crypt cells was obtained by mucosal scraping of the remaining jejunum. Enterocyte fraction position along the CVA was proven histologically, by noting decreasing invertase and alkaline phosphatase activities in sequentially isolated fractions and by demonstrating that [^{3}H]-methyl thymidine uptake was mainly in the final fraction of the lower villus-crypt cells. Brush border membrane vesicles (BBMV) were prepared from the upper villus, mid-villus and lower villus/crypt fractions using differential centrifugation and divalent ion precipitation. D-glucose uptake from each fraction showed a Na$^+$-gradient dependent time-course "overshoot", with linear uptake to 15 sec and a subsequent decline to a steady-state plateau. Varying D-glucose concentrations from 50-1000 μM demonstrated saturation kinetics of uptake with maximal transport rates (V_{max}) and Michaelis affinity constants (K_m) varying between fractions. The K_m was lowest in the upper villus as compared to the mid-villus and lower villus-crypt fractions. The V_{max} was lowest in the upper villus fraction, highest in the mid villus fraction and intermediate in the lower villus\crypt fraction. A linear relationship existed between LA concentration (25-200 μM) and uptake in each fraction. LA uptake was equivalent in all fractions when expressed per mg protein of the BBMV preparation. When expressed in terms of the BBMV surface area, uptake was greater in the upper villus than in the lower villus-crypt fractions. Thus, BBM uptake of both LA and glucose varies along the CVA. Passive uptake was greater, per unit surface area, in the upper villus than lower villus suggesting that the lipid permeability of the BBM is greater in the upper villus than in the lower villus or crypt.

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CHAPTER 1

INTRODUCTION

The ability to absorb and utilize nutrients is intrinsic to an organism's survival. In the case of mammals, this function is accomplished in the small intestine. The absorptive function of the small intestine is carried out by the small intestinal enterocytes. These epithelial cells cover the surface of the small intestinal villi which project into the intestinal lumen. They are polarized cells with a lipid bilayer brush border on their luminal pole. The absorptive surface area of the luminal brush border is increased by the presence of microvilli which project from the cell surface into the intestinal lumen. Embedded within the lipid bilayer of the microvillus or brush border membrane are the enzymes and proteins that enable the enterocyte to digest and absorb the organism's nutrients.

Enterocytes originate in the crypt of the villus and, as they mature, they advance along the crypt villus axis (CVA), finally reaching a point of extrusion at the tip of the villus from which they are sloughed into the intestinal lumen. During this advance, enterocyte morphology changes with the cells changing from being cuboidal to columnar type cells. As well, their microvilli change from the broad and relatively sparse microvilli of the crypt cells to the narrower and more numerous microvilli of the upper villus enterocytes [1]. The length of the microvilli increases as enterocytes advance and mature along the CVA, with lengths of 1.8μm in microvilli from enterocytes from the upper villus in the rat, as compared to values of 1μm and 0.8μm in middle and lower villus enterocytes [2].

As enterocytes advance along the CVA, the intra cellular activity of thymidine kinase (TK) decreases while the brush border membrane activities of alkaline phosphatase (ALP) and invertase (INV) increase [3-12]. This increased activity of hydrolytic enzymes in the upper villus is consistent with the role of the upper villus in nutrient digestion and absorption. Conversely, the higher concentration of TK, which is involved in cell synthesis in the crypt cells, reflects the role of crypt cells in cell replication.

The absorption of nutrients from the intestinal lumen by the enterocytes has been studied. Following a meal rich in dietary fats, lipid droplets are seen predominantly in upper villus absorptive cells, suggesting that uptake of lipid occurs mainly in the upper villus [13]. Autoradiography studies have confirmed this finding by demonstrating that uptake of palmitic acid is predominantly in the upper villus segment of the CVA [14]. The uptake of hexoses and amino acids along the CVA has also been studied using autoradiographic techniques which confirm that uptake of these nutrients also occurs predominantly in the enterocytes of the upper third of the villus [15-18].

Thus, it appears that nutrient uptake occurs mainly in the upper villus. In support of this concept, in vitro studies using brush border membrane vesicles (BBMV) prepared from enterocyte fractions harvested from along the CVA have established that there is greater uptake of amino acids and hexoses in the upper than in the lower portions of the CVA [10,4,11,19]. Indeed, it has been suggested that a second hexose carrier may appear as the enterocytes migrate along the CVA [11,4,20-22], and that the kinetic properties of carrier-mediated transport vary along the CVA [10,4,11]. Taken together, these findings give strong support to the concept that nutrients are absorbed principally in the upper portion of the villus.

Although the intestinal Na^+-dependent glucose transporter has been cloned [23-27], the kinetic characterization of intestinal glucose transport remains focused on the estimation of the maximal transport rate (V_{max}), the Michaelis constant (K_m), and the passive permeability coefficient (Pd). Theoretical and experimental considerations have demonstrated the importance of correction for the intestinal unstirred water layer (UWL) resistance [28]. Using isolated BBMV eliminates paracellular movement and minimizes the effective resistance of the UWL, but there remains considerable variability in the magnitude of the estimates of V_{max} and K_m dependent on the methods used to estimate them [29]. Linear transformations of the Michaelis-Menten equation may not always produce valid estimates of V_{max} and K_m, particularly in the presence of an UWL and when the magnitude of the passive component is not taken into account [30-33]. The non-linear methods of estimating V_{max} and K_m are variable; these include the use of the statistical software programs Enzfitter® and Systat®, with variable methods of weighting, as well as the method used by Karasov and Diamond [34] in which a substrate concentration several times above the value of the K_m is used to estimate V_{max} after correction for the contribution of passive permeation using the uptake of L-glucose. While it may be argued that it is appropriate to use any of these linear or non-linear methods to estimate kinetic constants under experimental conditions where one is comparing treatment A with treatment

B, a change in the contribution of passive permeation or in the resistance of the UWL may produce apparent differences, where in fact the V_{max} or K_m do not change, or may obscure demonstration of differences where such differences do, in fact, exist. In addition, differences may result from the use of parametric versus non-parametric methods to estimate the magnitude of V_{max} and K_m (35,36). Furthermore, the lack of consensus for the optimal method of estimating these kinetic constants results in difficulty comparing experimental results obtained in one laboratory using one method and those obtained in another laboratory using a second method. Accordingly, we chose to analyze the kinetic data for glucose uptake (V_{max} and K_m) with several methods of analysis.

BBMV uptake data is usually normalized to unit protein. When using protein as the denominator, it is assumed that the proportion of glucose carrier to total membrane protein remains constant along the CVA. Morphological data suggests that the number of glucose carriers per unit membrane surface area remains constant from crypt to villus tip [2], but it is unknown whether this is the case for the membrane fatty acid binding protein which is thought to play a role in the uptake of fatty acids, particularly at low concentrations [37]. In addition, the BBM surface area becomes amplified as the enterocytes migrate up the CVA [2], so that the appearance of greater uptake of nutrients from the upper than the lower portion of the CVA may not necessarily be from a greater true V_{max} of individual glucose carriers per unit BBM surface area from upper villus cells, but instead may be due to a greater total surface area. For this reason, it may be necessary to use other denominators of uptake.

BBMV have been shown to be a useful tool to assess the uptake of LA by the small intestine [38,39]. However, no published studies are available comparing the uptake of this nutritionally essential long chained fatty acid into BBMV obtained from enterocytes originating at different levels of the CVA. Accordingly, these studies were undertaken to test the null hypothesis that there is no gradient in the uptake of LA or D-glucose along the CVA of rabbit jejunum. The results suggest that the null hypothesis is rejected, and that the passive permeability properties for LA and the V_{max} and affinity constant K_m for glucose uptake are greater for the BBM of enterocytes from the upper as compared to the lower portions of the CVA.

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CHAPTER 2

SMALL INTESTINAL DEVELOPMENT AND DIFFERENTIATION

2.1 INTRODUCTION

In the prenatal period, nutrition is provided to the mammalian fetus transplacentally. At this stage of development, the small intestine does not play a role in nutrition. However, following birth, transplacental nutritional support ceases and the small intestine must adapt to its role as the locus of nutrient absorption in order for the organism to survive.

During the perinatal and infant stages of development, the gut must absorb a diet consisting of only one substrate: milk. Weaning constitutes a major adaptational challenge in the mammal's lifespan as the growing mammal's diet changes from milk to a diet consisting of both milk and the diet it will follow in adulthood and finally an adult diet which, in all species except man, is devoid of milk.

In this chapter, the patterns of prenatal and postnatal development of the small intestine will be reviewed. The morphology and physiology of the gut during the different stages of development will be examined and the changes occurring with maturation will be reviewed. The response of the gut to changes in the nature of the organism's nutrition will be examined.

2.2 FETAL AND PERINATAL DEVELOPMENT

In an autoradiographic study of fetal rats, which are born on the 22nd day of gestation, Hermos et al [1] noted formation of villi at the 17th day of gestation. By the 19th day of gestation, tall, well developed villi in the proximal intestine are present. In contrast, well defined crypts do not develop until shortly after birth. Using a [^{3}H] thymidine autoradiography technique, it was demonstrated that, in the fetal rat, DNA synthesis and cell proliferation occur in epithelial cells along the entire length of the villi. However, following birth, cell replication is limited to the crypts.

Using autoradiographic techniques, Smith [2] established that Na^+ dependent uptake of alanine in the newborn and one day old pig occurred along the entire crypt-villus axis (CVA). In contrast, the adult rabbit intestine only had uptake in the villus tips. It was postulated that this alteration in the pattern of amino acid uptake in adult small intestine occurs as a result of changes in the postnatal pattern of cell replacement and differentiation. Uptake along the entire CVA in the newborn animal may be secondary to early maturation of transport capacity coupled with normal cell migration along the CVA axis or negligible cell migration coupled with slow maturation of transport processes.

Intestinal nutrient transporter development begins in the prenatal period. As reviewed by Buddington and Diamond [3], transporters do not appear until the intestinal epithelium itself has differentiated to form crypts, villi and microvilli. Glucose and amino acid transporters appear prenatally while bile acid and fructose transporters appear only postnatally. Guinea pigs express transporters early in the second half of gestation while rats and rabbits express transporters only towards the end of gestation.

In summary, differentiation of enterocytes along the CVA does not occur until the post partum period. Prior to birth and immediately thereafter, cells of the CVA exhibit both a proliferative and nutrient absorptive capacity. However, following birth, cells in the crypt assume the role of cell replication while the cells of the villus column assume a nutrient absorptive role.

2.3 POSTNATAL DEVELOPMENT

Following birth, nutrition is provided to the suckling animal in the form of milk. Milk provides the developing organism with a diet that is high in fat and low in carbohydrates and iron as compared to the adult diet. Carbohydrates are presented in the form of lactose, in contrast to the adult diet where maltose, isomaltose and invertase constitute the principal carbohydrates. The relative proportion of fats, carbohydrates and proteins also changes as the organism's diet switches from milk to an adult diet. These changes are dependent on whether the adult animal assumes a high protein carnivorous diet or a high carbohydrate herbivorous diet.

In the postnatal period, significant growth occurs in the small intestine. Smith and Jarvis [5] measured the microscopic and macroscopic aspects of growth in the pig small intestine in the perinatal period. During the first 10 days of postnatal life, the length and diameter of the small intestine increases 80% and 30% respectively. The absorptive area of the small intestine doubles during this period. Villi formed at birth are 29, 50 and 75% longer by day 10 in the proximal, mid and distal segments of the small intestine respectively. Maximal elongation of villi occurred on day six of postnatal life. Mitoses in the postnatal pig are confined to the crypts, with time taken for complete cell replacement along the villus of 19 days.

Koldovsky et al [6] have studied the rates of proliferation and migration of intestinal epithelial cells in rats. Animals were injected intraperitoneally with [^{3}H] thymidine. In rats older than 28 days, two hours after injection, only epithelial cells in the crypt were labelled. By 24 hours, labelled cells occupied 40-55% of the height of the villus, and, after 48 hours, labelled epithelial cells had reached the tips of the villus. In contrast, in the intestine of animals aged 7, 11 and 12 days, two hours after radio-isotope injection, labelled cells were found in the crypts. After 24 hours, labelled cells were still predominantly in the crypts and occupied the basilar 10% of the villi while, at 48 hours post labelling, only 25% of the villi contained labelled cells. Thus, enterocyte migration in the small intestine of the suckling animal proceeded at a rate 20% or less than that of the weaned animal. This rate of migration was similar in the duodenum, jejunum and ileum of the suckling animal. As well, the intestinal crypts of the suckling animals contained fewer cells than those of the adult, and the cells of the villi in the suckling animal contained an increased density of [^{3}H] thymidine grains suggesting a lower rate of cellular proliferation and division in the suckling as compared with the weaned animals.

With weaning, the nutrient transporter and enzyme activities of the brush border membrane (BBM) change. As dietary sugars change from lactose to maltose, isomaltose, fructose and sucrose, lactase activity declines and invertase and isomaltase activity increases as does fructose and bile acid transport. The animal, which previously had a diet of one substrate (milk), adapts to a varying diet in terms of composition, quantity and frequency of feeding [3].

The BBM profile of nutrient transporter activity varies according to the dietary content of the animal. Fructose transport capacity following weaning is low in cats which are carnivorous and do not encounter fructose in their diet. By contrast, fructose transport capacity in the rat small intestine increases with age as fructose assumes a role post weaning in the rat's diet [7].

Just as the nutrient transporter and enzyme activity profile of the BBM change with weaning, so do the lipid permeability, physical properties and chemical composition of the membrane. Schwarz et al [8] analyzed the cholesterol and phospholipid content and fluidity of the BBM in rabbits during the suckling period, during weaning and in adulthood. They demonstrated that significant increases in the cholesterol to phospholipid content ratio occurred with weaning associated with a decline in membrane fluidity. Jejunal BBM fluidity exceeded that of the ileum at all ages studied. They suggested that changes in fluidity may relate to maturation of enterocyte transport function. Possibly, lipid fluidity changes were associated with changes in the efficiency of carrier-mediated amino acid and glucose transport.

In a study of rats between the ages of 9 and 25 days, Meddings and Theisen [9] established that as the rat's diet changes from maternal milk to a diet of milk plus chow and finally chow alone, the BBM lipid permeability, dynamic fluidity and hydrophobicity decrease. It was postulated that these changes were physiologically significant. High lipid permeability in the rat prior to weaning allows rapid absorption of the large amounts of triglycerides in maternal milk. With a change to a carbohydrate rich diet following weaning, membrane permeability decreases in response to the reduced dietary content of triglycerides.

The signals affecting these changes are not yet understood. Thyroxine and cortisone have been incriminated as modulators of postnatal intestinal differentiation. In two studies published in 1991, Yeh et al [10,11] examined the effects of thyroxine and cortisone on jejunal invertase and lactase expression in the early postnatal period. Lactase activity, essential for the digestion of milk, is highest in the immediate postnatal period. It subsequently declines as milk's role in the animal's diet declines. The administration of cortisone resulted in decreased lactase activity and cell turnover rate. Given alone, thyroxine did not induce changes in lactase activity. However, when given with cortisone, there was a dramatic decrease in lactase activity associated with a markedly increased cell turnover rate. As well, cortisone caused invertase activity to appear earlier and more prominently in postnatal rats. This effect of cortisone was enhanced by co-administration of thyroxine. The authors concluded that cortisone and thyroxine cooperatively stimulated invertase expression and reduced lactase activity during early postnatal life. They postulated that this effect occurred at the posttranslational level.

The role of nutrition on modulating intestinal maturation has been examined. Castillo et al [12] examined the role of intraluminal nutrients in regulating intestinal maturation. In a group of suckling rats, they discontinued all oral feedings and began parenteral nutrition. They noted a decreased intestinal length, mucosal weight, DNA protein and total disaccharidase activity in these animals as compared to normally weaned controls. When oral nutrients were reintroduced to these parenterally fed animals, all variables except total lactase activity returned to the values found in normally weaned, age matched controls. These results suggested that the changes brought on by the absence of intraluminal nutrients were not permanent and rapidly corrected upon refeeding.

In 1968, Ginsburg et al [13] reported that rats fed carbohydrate free diets starting 10 days post partum demonstrated intestinal glucose uptake rates 75% of that exhibited by rats maintained on 60% glucose diets when uptake was measured 8 to 18 weeks later. However, rats initially fed low carbohydrate diets and switched to 30% glucose diets at 8 weeks of age exhibited glucose uptake 180% that of rats maintained on carbohydrate free diets and 134% that of rats maintained on 60% glucose diet. Thus, a late effect of early feeding of a high carbohydrate diet was demonstrated.

In 1985, Karasov et al [14] postulated the existence of critical-period programming in intestinal nutrient uptake. They defined critical-period programming as a biological mechanism which is irreversibly turned on or off only once during an organism's lifespan in response to conditions prevailing at a critical stage in the organism's development. They suggested that the composition of a nutritionally adequate ration early in life has lasting consequences for the nutrient transport mechanisms of the animal's intestine or for the intestine's ability to turn transport mechanisms on or off adaptively later in life.

They acknowledged that Ginsberg et al's [13] study suggested possible critical period programming occurring in the rat small intestine. However, they believed that the experimental design used in this study was flawed. To determine whether nutrient active transport in the small intestine was subject to critical period programming, they compared in vitro amino acid and glucose transport in two groups of mice: one group maintained on a life long high-carbohydrate, maintenance protein diet and a second group maintained on a life long high protein, carbohydrate free diet. They were unable to demonstrate the presence of irreversible differences in nutrient uptake in these two groups thus refuting the existence of critical period programming in the uptake of amino acids and glucose in the mouse small intestine.

Other authors have examined the possibility that critical period programming exists in the small intestine as a response to different nutrient exposure in early stages of development. Thomson and Keelan [15] examined the effect of administering cholesterol early in life to rabbits on the subsequent passive and active uptake of nutrients by the small intestine. Early exposure to a high cholesterol diet results in increased jejunal passive uptake of octanoic, lauric, cholic and taurocholic acid, increased active uptake by the jejunum of L-leucine and increased active uptake by the ileum of D-glucose, D-galactose and L-leucine as compared to animals maintained on standard rabbit chow diet. These changes were present 14 weeks after the animals were switched to a regular chow diet after being on the high cholesterol diet only 4 weeks. As well, animals fed a high cholesterol diet at an early age exhibited different uptake patterns when reexposed to a high cholesterol diet as adults than rabbits exposed to a high cholesterol diet for the first time as adults. Early changes in the dietary content of cholesterol may have prolonged effects on intestinal transport function and early exposure to cholesterol may alter the subsequent intestinal adaptive response to dietary cholesterol exposure, suggesting critical period programming.

Thomson et al [16] subsequently showed that varying the dietary fatty acid composition early in life influenced the intestinal passive and active absorption characteristics later in life. Rats weaned onto a semisynthetic polyunsaturated fatty acid enriched diet demonstrated decreased small intestinal uptake of glucose as compared to rats maintained on a semisynthetic saturated fatty acid enriched diet. It was postulated that these changes were secondary to dietary induced changes in the activity of intestinal lipid-metabolizing enzymes which alter fatty acids incorporation into membrane phospholipids. This modification in turn alters the physical or chemical properties of the brush-border membrane resulting in modified active or passive nutrient transport and may thus represent critical period programming of nutrient uptake in the small intestine.

In summary, the postnatal period is a time of significant growth in the mammalian small intestine as it assumes the role of nutrient absorption. Maturation is associated with an acceleration in cell replication and movement along the CVA from the newborn to perinatal to adult animal. As the mammal's diet changes from a milk diet to its adult diet, BBM nutrient transporter and enzyme activity levels change to adapt to the dietary change. In addition, in response to changes in dietary content of triglycerides, BBM lipid permeability changes. The signals modulating these developmental changes are poorly understood. Hormones, such as thyroxine and cortisol, may play critical roles in small intestinal development. Absorptive capacity of the gut may be modulated by the nature of the oral intake. These changes may be reversible. However, critical period programming may occur in the small intestine such that absorptive capacities of the small intestine may be irreversibly modulated by early life exposure to specific nutrients.

2.4 MORPHOLOGY AND FUNCTION IN THE ADULT

In 1962, Padykula [17] reviewed the functional interpretations of intestinal morphology. Small intestinal absorptive cells originate in the crypts, migrate upwards along the villus and move steadily towards the villus tip, being extruded at a specific site termed the "extrusion zone". Associated with this migration is morphologic and histochemical evidence of progressive differentiation of the cells. Undifferentiated crypt cells have a high concentration of ribonucleoprotein which decreases as they advance towards the villus tip. As well, morphologically, cells change as they advance along the CVA, becoming taller with a more conspicuous Golgi region and decreased cytoplasmic basophilia. At the villus tip, cell cytoplasm is predominantly acidophilic.

Brown [18] used electron microscopy to examine the BBM of epithelial cells of the small intestine. The microvilli in the crypt cells were short, wide and relatively few in number. As epithelial cells derived from progressively more distal along the crypt villus axis were examined, villi became progressively taller, thinner and more numerous, associated with a corresponding increase in the surface area of the microvilli, along with a decrease in the volume of the absorptive cells.

Just as morphological differences along the CVA have been described, so have differences in structure and enzymatic profile of cells along the crypt villus axis been noted. Using horizontal sectioning of fresh frozen rat intestine, Nordstrom et al [19] demonstrated alkaline phosphatase (ALP), dipeptidase and disaccharidase activity along the villus but not in the crypts. The highest activities of these enzymes were in the apical halves of the villi with ALP activity being more distinctly located at the tips of the villi.

Using an in vitro technique of cell fractionation, Weiser [20] harvested cells from different levels of the CVA. He established that ALP activity was maximal at the villus tip while invertase (INV) activity was maximal in the distal villus and declined slightly at the tip. Both of these enzymes had minimal activity at the crypt level, while thymidine kinase (TK) activity was noted only in the crypt segment and proximal villus cell fraction.

The method devised by Weiser [20] to isolate cells from along the CVA and confirm their origin by determining their enzyme profile has been used, with modifications, by many authors [21,22,23]. In Weiser's study [20] in which female Sprague Dawley rats were used, enzyme activity was expressed against a denominator of percent total cells isolated, with higher percentages representing fractions isolated from more proximal along the CVA. Nine cell fractions were isolated from along the CVA. Maximal ALP activity was recorded in the initially isolated fraction with negligible activity present in the final 2 fractions. By contrast, INV activity was maximal in the second isolated fraction and decreased distally towards the villus tip and proximally towards the crypt with negligible activity in the final isolated fraction. TK activity became significant after the 4th fraction, reaching maximal activity in the final fraction. All fractions were isolated by agitation of intestinal segments without scraping of the remaining intestine to harvest the crypt cells.

Freeman et al [21] isolated 9 fractions from along the CVA, without using a mucosal scraping procedure for the final fraction. Maximal INV (results not presented) and ALP activity occurred in the earliest fractions with only 30-40% of these initial activities detected in later fractions. TK activity was highest in later fractions and least in initial fractions. Enzyme activity was not expressed in absolute terms but rather the activity in each fraction was expressed in terms of the percentage of the highest value in each assay.

Meddings et al [22], using male New Zealand white rabbits, harvested only three fractions, with the final fraction obtained from scrapings of remaining small intestine mucosa. He established that maximal INV activity occurred in the initially isolated fraction with less activity in the intermediate segment and minimal activity in the mucosal scrapings segment with activities, expressed in terms of units/mg protein, of 0.067, 0.058 and 0.005 respectively. TK activity was highest in the mucosal scrapings segment with activities, expressed as umol/min/mg protein, of 3.9 in the initially isolated fraction, 5.4 in the intermediate fraction and 11.8 in the mucosal scrapings fraction.

Dudeja et al [23] demonstrated, in male Lewis rats, a gradient of INV activity with maximal activity occurring in the villus tip segment and least activity in the lower villus segment with values, expressed as umol activity-mg protein^{-1}-min^{-1}, of 3.66 in the villus tip segment, 1.63 in the mid villus and 1.23 in the lower villus of non diabetic rats. [^{3}H] Thymidine uptake, as a measure of TK activity, expressed as counts-min^{-1}mg protein^{-1}x 10^{3}, was 10.0 at the villus tip as compared to 17.6 at the mid villus level and 54.7 at the lower villus. Mucosal scraping was not used to isolate the final fraction.

These studies all demonstrate a gradient of ALP, INV and TK activity along the CVA. However, the techniques, units of measurement and species of laboratory animal used in each study were different, making comparisons of the results difficult if not impossible. As well, the relative ratios of enzyme activity between initial, middle and final isolated fractions were different. Thus, any comparison between data obtained from these studies is likely to be biased.

The importance of the BBM lies in its pivotal position between the intestinal lumen and the cytoplasm of the enterocyte. Prior to the early 1970's, it was not possible to study the BBM in isolation from the enterocyte. However, in 1973, in a landmark paper, Hopfer et al [24] described a method, using differential centrifugation and divalent ion precipitation of intracellular organelles, of isolating purified BBM in vesicular form prepared from a homogenate of rat jejunal and ileal mucosal scrapings. In this study, the authors demonstrated that the isolated BBMV retained intact the Na^+ dependent D-glucose transport system with D-glucose transport into the intravesicular space, thereby confirming the validity of their isolation technique.

Hopfer's isolation technique has been applied to the study of BBM composition and intestinal transport processes. The BBM consists of a cholestrol-phospholipid bilayer with an exofacial (outer) and cytofacial (inner) leaflet. Interspersed throughout the bilayer are proteins which serve as enzymes, such as the disaccharidases and nutrient transporters, such as the Na^+ dependent D-glucose transporter. Studies have focussed on the composition of the lipid bilayer and influences membrane structure may have on BBM protein function.

The term membrane fluidity has been used to describe the relative motional freedom of lipids within the lipid-bilayer. As reviewed by Schacter [25], lipid molecules within the lipid bilayer have three basic patterns of motion: transverse diffusion from one hemileaflet to another, rotational diffusion around an axis perpendicular to the plane of the bilayer and lateral diffusion in the plane of the hemileaflet. Fluidity can be defined as the weighted sums of these three types of motion with increased fluidity corresponding to increased lipid molecular movement.

Methods have been devised to quantify membrane fluidity. Probe independent techniques include calorimetry, X-ray diffraction, electron diffraction light scattering and certain applications of nuclear magnetic resonance. Probe dependent spectroscopic techniques utilizing fluorescence, electron spin resonance and nuclear magnetic resonance have proven superior in the study of biological membranes due to technical limitations of probe independent techniques. Fluorescence polarization and excimer fluorescence intensity techniques have proven particularly useful in the assessment of membrane lipid fluidity [25].

Studies of BBMV prepared from small intestinal mucosal scrapings have demonstrated that fluidity of the lipid bilayer changes with age [26] and is lower in the ileum than the jejunum [27]. Study of BBMV prepared from enterocytes harvested from different levels along the CVA have also determined that fluidity changes with enterocyte maturation and differentiation.

Using New Zealand white rabbits, Meddings et al [22] demonstrated that BBM fluidity decreases with migration from the crypt to the villus tip. The Na^+ dependent D-glucose transporter in BBMV prepared from crypt enterocytes had a higher affinity to D-glucose than the transporter in the villus tip derived enterocytes. This distinction disappeared when differences between the lipid environments of the vesicle preparations were removed. They postulated that decreased fluidity and the resultant increased rigidity of upper villus BBM impeded the conformational change of the D-glucose transporter in response to Na^+ binding required for the subsequent binding and transport of D-glucose. Thus, the transporter in this circumstance had less affinity for D-glucose.

Contrary to these findings were the results of Dudeja et al [23]. In their studies, they used the Lewis rat and determined that, rather than decreasing from crypt to villus tip, fluidity of the brush border membrane increased from the crypt to the villus tip. Despite this, D-glucose uptake showed a similar profile in the nondiabetic rat as demonstrated in the study of Meddings et al. Dudeja et al thus suggested that fluidity gradients of the BBM during migration along the CVA are species-dependent and do not influence Na^+ dependent D-glucose transport.

Other researchers have examined the role of membrane fluidity in BBM function. Dudeja et al [28] demonstrated that increasing the in vitro fluidity of the exofacial leaflet of the BBM of rats by the addition of benzyl alcohol decreased membrane leucine aminopeptidase activity without affecting maltase, INV, ALP or gamma-glutamyltranspeptidase activities while fluidizing the cytofacial leaflet increased Na^+ dependent D-glucose transport without affecting Na^+ dependent L-leucine transport. The authors suggested that alterations in lipid fluidity appear to have important and relatively selective physiological effects. However, they suggested further studies were needed to rule out whether changes, other than fluidity, in the physical state of the lipids in the hemileaflets could have caused these changes in enzyme and transport activity.

This conclusion was consistent with a previous study from the same laboratory [29]. In this study, Na^+ dependent D-glucose uptake by BBMV prepared from a homogenate of jejunal mucosal scrapings obtained from rats fed a nutritionally complete diet enriched either in unsaturated (fish oil) triacylglycerols or saturated (butter fat) triacylglycerols was determined. Na^+ dependent D-glucose uptake demonstrated a higher maximum velocity (V_{max}) but similar affinity constant (K_m) in BBMV prepared from rats fed a fish oil enriched diet as compared to rats fed a diet enriched in butter fat. Membranes prepared form animals fed a fish oil enriched diet possessed a higher percentage of (n-3) unsaturated fatty acids and saturated fatty acids and a lower percentage of monounsaturated and (n-6) fatty acids than animals fed a butter fat enriched diet. The authors speculated that changes in the fatty acid composition of the particular domain of the D-glucose transporter might be responsible for the increased D-glucose transport seen in the rats fed a fish oil enriched diet.

Thus, the role of fluidity in membrane function has not been clearly established. Potentially, changes in the composition of the hemileaflets of the BBM, of either diet or hormone mediated origin, may have the effects both of altering the lipid domain of the membrane proteins and thereby altering their activity, by an as yet undetermined mechanism, as well as altering membrane fluidity. In this case, the change in membrane fluidity per se is not the cause for altered protein function. On the other hand, Meddings' theory of membrane fluidity being the principal cause of altered membrane function has not been disproven. Further investigations will be necessary to determine this issue.

In summary, enterocyte migration along the CVA is marked by changes in enterocyte morphology as the cells differentiate from the cuboidal cells of the crypt with sparse microvilli to the columnar villus enterocytes with profuse microvilli at the villus tip. Functionally, enterocytes acquire different enzyme profiles as they advance along the crypt-villus axis with increasing activity of digestive brush border enzymes such as ALP and INV and decreasing activity of TK, to accomodate the change in role from replicative to absorptive cells. BBM fluidity also changes along the CVA. However, these changes appear to be species specific and their significance in determining nutrient uptake and membrane function have not been determined. Nutrient uptake by BBM transporters may be influenced by the fatty acid domain of the transporters rather than membrane fluidity.

2.5 NUTRIENT UPTAKE ALONG THE CRYPT-VILLUS AXIS

Glucose uptake along the CVA has been studied [21,22,23]. In all three studies, Na^+-gradient-dependent D-glucose uptake was noted in BBMV prepared from enterocytes obtained from each level of the CVA. Maximal uptake occurred in vesicles prepared from enterocytes harvested from the upper villus. While Freeman et al [21] only prepared vesicles from enterocytes isolated from the upper villus and crypt compartment, Dudeja et al [23] and Meddings et al [22] prepared 3 fractions of vesicles derived from upper villus, mid villus and crypt enterocytes. All authors suggested that more than one Na^+-gradient-dependent D-glucose carrier was present along the crypt villus axis. Freeman et al [21] suggested that a single D-glucose carrier was present in crypt epithelial cells while two or more D-glucose carrier systems were present in villus epithelial cells. Dudeja et al [23] postulated that the lower villus compartment BBM contained a high affinity-low capacity D-glucose carrier while the mid-villus and villus tip compartment BBM possessed a low affinity-high capacity D-glucose transporter. Meddings et al [22] also suggested the possibility of two BBM D-glucose transporters: a high affinity, low capacity D-glucose carrier in the crypt compartment and a low affinity high capacity D-glucose carrier in the mid-villus and upper-villus compartment. However, they also suggested that differences in D-glucose uptake by the BBM along the CVA could be secondary to the effects of a changing lipid environment in the membrane on a single D-glucose transporter (see above).

Lipid movement across the BBM at different levels of the CVA has not been as intensively studied as the movement of D-glucose. It has been suggested that lipid movement across the BBM is passive [30,31]. However, carrier mediated lipid uptake by the BBM has been described with identification of a long chained fatty acid [32,33] as well as cholesterol and phosphatidylcholine carrier protein [34,35,36] located within the BBM.

In support of this concept, an overshoot of linoleic acid (LA) uptake over time in BBMV prepared from a homogenate of jejunal and ileal scrapings has been reported [37]. Partial inhibition of LA uptake by phloretin and uptake of LA into the intravesicular space were also recorded. The authors suggested that absorption of LA in vivo could be partially due to a carrier mediated facilitated transport mechanism.

In a later study, Keelan et al [38] confirmed that phloretin inhibits LA uptake by BBMV. They were, however, unable to demonstrate an overshoot of LA uptake over time or evidence of intravesicular accumulation of lipid. This study therefore demonstrated that BBM uptake of LA was passive. However, they were unable to disprove or confirm the possibility that an energy-dependent or protein-associated component of LA uptake by the BBA existed.

Uptake of lipids is predominantly at the level of the mid and upper villus, as evidenced by autoradiographic studies conducted by Haglund et al [39] on the intestinal absorption of palmitic and oleic acid. Earlier studies supported this concept by demonstrating that, following a lipid meal, the apical cytoplasm of enterocytes in the upper third of the villus are packed with lipid droplets while cells nearest the crypt are generally free of lipid [17].

Thus, in conclusion, as the mammal matures, significant changes occur in the morphology and function of the small intestine. Function becomes compartmentalized with cell replication occurring in the crypt and nutrient uptake occuring along the villus column, predominantly in the mid and upper villus segments. In contrast, both replication and nutrient uptake in the newborn animal occurs along the entire CVA.

In summary, in the mature animal, nutrient absorption across the BBM occurs principally in the upper portions of the CVA. In the case of Na^+-dependent D-glucose transport across the BBM, it has been postulated that increased uptake in the upper villus occurs secondary to the development with maturation of different D-glucose transporters in the villus as compared to the crypt. The uptake of lipids along the CVA has not been as well studied as uptake of D-glucose. Autoradiographic and histological studies suggest that lipids, much like D-glucose, are preferentially absorbed in the upper villus. A fatty acid binding protein has been found in the BBM. As well, studies have shown that lipid uptake by the BBM can be inhibited. Thus, it is possible, lipid uptake by the enterocyte occurs by both passive and active mechanisms.

2.6 OBJECTIVE OF CURRENT STUDY

The in vitro uptake of glucose [21,22,23] by BBM harvested from along the CVA has been studied. However, the in vitro uptake of lipids by the BBM along the CVA has not been reported. The development of methods for studying lipid uptake along the CVA would facilitate the determination of the effects of different diets or hormonal manipulation of the animal's internal milieu on lipid uptake at different levels of the CVA. It would allow study of uptake in isolation from the effect of the unstirred water layer which serves as an interface between the BBM and the intestinal lumen.

The objective of this study is to establish a method of sequentially harvesting enterocytes from the upper villus to crypt and isolating them in fractions from which BBMV can be prepared. The technique will be verified by demonstrating a gradient of decreasing ALP and INV activity and increasing TK activity in the sequentially isolated cell fractions, from villus tip to crypt and by histologically confirming the sequential removal of enterocytes from along the CVA with successive fractions.

Once the cell fractionation technique has been verified, BBMV will be prepared from each fraction. The purification of vesicles will be confirmed by demonstrating an increase in INV and ALP activity in the vesicle preparation as compared with activity in the cell fractions. The structural integrity of the BBMV formed will be confirmed by demonstrating an overshoot in Na^+-dependent D-glucose transport over time into the vesicles. Once it has been confirmed that the technique utilized is capable of isolating intact vesicles selectively from along the CVA, the uptake of LA as a function of varying times of incubation and concentration of LA will be investigated.

The null hypothesis in this study is that in the absence of an unstirred water layer and subcellular components of the enterocytes, uptake by BBMV of LA will be passive and equal in vesicles harvested from along the CVA.

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CHAPTER 3

SMALL INTESTINAL ADAPTATION

3.1 INTRODUCTION

In order to survive, an organism must be able to adapt to a changing environment. It must adapt to variations in both the availability and composition of nutrients. As well, it must adapt its utilization of foodstuffs to changes in its internal milieu, such as occurs during pregnancy, illness and aging. The optimal utilization of foodstuffs is critical to survival.

The ability of the small intestine to modify its functional capacity and structure in response to changes in its external or internal milieu has been termed small intestinal adaptation. In this chapter, the concept of small intestinal adaptation will be examined. The response of the small intestine to changes in the character and quantity of nutrients will be examined as well as its response to a changing internal milieu brought on by hormonal changes such as occur with lactation, pregnancy and diabetes mellitus (DM) as well as changes occurring with radiation therapy, senescence and small intestinal resection. Experimental models that have been developed to examine these changes will be reviewed and possible signals initiating these changes will be discussed.

3.2 SMALL INTESTINAL RESPONSE TO RESECTION AND LUMINAL NUTRITION

In 1912, Flint [1] reported on a study in which he resected up to 80% of the small intestine in dogs. In animals surviving the procedure, a characteristic pattern of diarrhoea and weight loss followed by recovery was noted. In the recovery phase, histological examination of the remaining intestine of these animals demonstrated an increase in the portion of the mucosa forming the crypts to a size equal to that of the villi. As well, irregularity of the shape of the villi as compared to those of control animals was noted.

In a more recent study, Freeman et al [2] assessed the functional as well as morphological changes in the distal small intestine of rats undergoing a 66% proximal jejunoileal resection. In rats undergoing such a resection and fed rat chow post operatively, mucosal weight, protein and DNA content were increased in the remaining ileum as compared to control rats that underwent small bowel transection and reanastomosis without resection. Increased villus and crypt heights with increased mitotic indexes in the crypts were noted in animals undergoing resection. Negligible Na^+ dependent D-glucose transport was noted in the ileum of the control rats. However, transport in the remaining ileum of resected rats increased significantly with the appearance of two Na^+ dependent D-glucose transport systems: one a low-affinity, high capacity transporter and the second a high-affinity, low-capacity transporter. Thus, adaptation to bowel resection involves increased cell replication in the remaining small intestine associated with the development of active nutrient transport in segments of the small intestine in which nutrient transport did not previously occur.

The pivotal role of luminal nutrition in preserving normal small intestinal structural integrity and in stimulating intestinal growth and adaptation has been examined. Levine et al [3] studied the disaccharidase activity and small intestine mass in two groups of rats: one group which was intravenously fed an elemental diet (consisting of 30% dextrose, 5% amino acids, electrolytes and vitamins) post surgery and a second group which received the same solution orally. After 1 week, mucosal height, gut weight, mucosal protein and maltase, invertase and lactase activities were studied in each group and were significantly less in the group receiving parenteral nutrition. This study thus demonstrated the critical role of luminal nutrition in maintaining intestinal mucosal integrity.

The importance of oral nutrition in maintaining normal intestinal integrity was further demonstrated by Buts et al [4]. They studied the small intestine in rats and demonstrated that, in comparison to rats maintained on a standard rat chow diet, rats starved for 96 hours demonstrated mucosal hypoplasia, villus and crypt shortening in the jejunum while ileal crypt depths increased significantly with decline in ileal villus height, mucosal weight and DNA content relative to values in the control rats maintained on the standard diet. Although body weight declined by only 16-17% during this time, jejunal mucosal weight, DNA and protein content declined by 36-40% relative to values in the control rats. Refeeding produced recovery of the above noted changes.

Thus, luminal nutrition is important to the maintenance of normal small intestinal form and function. However, is luminal nutrition necessary for adaptation to occur following bowel resection? To answer this question, Levine et al [5] examined the role of luminal nutrition on small intestinal adaptation in rats following a 70 centimetre proximal small intestinal resection. Small intestinal hyperplasia occurred in the remaining small intestine in rats fed an elemental diet orally following resection with increased gut weight, mucosal weight, protein and DNA content and mucosal height of the intestine both proximal and distal to the line of resection relative to values in sham-operated orally-fed animals. An adaptive response did not occur in animals given the same elemental diet intravenously following bowel resection, with no difference being noted in gut weight, mucosal weight, mucosal protein or DNA between resected or sham-operated intravenously alimented rats. Thus, for a small intestinal adaptation response to occur following bowel resection, continued oral nutrition is required.

The mechanism(s) by which luminal nutrition influences small intestinal growth and function is unclear. Williamson et al [6] studied DNA specific activity in the jejunum of rats linked by vascular parabiosis (cross circulation) to rats undergoing either transection with reanastomosis or resection of the jejunum. All animals received a standard rat chow diet prior to and after surgery. Increased mid and distal small bowel DNA specific activity was noted in rats parabiotically linked to rats which had undergone bowel resection, suggesting that hyperplasia of the intact intestine in these parabiotically linked rats was occurring. However, DNA specific activity was higher in rats actually undergoing resection. These results suggest both local and systemic factors mediate intestinal adaptation following small intestine resection.

Studies have been carried out to identify the systemic factors mediating post resection small intestinal adaptation. Grey and Morin [7] demonstrated that, in rats which underwent a 50% proximal small intestinal resection and were fed orally following surgery, a heat-stable acidic extract of proximal small intestinal mucosa was present which stimulates DNA synthesis in mouse jejunal explants in culture. Gel filtration studies demonstrated that this growth-stimulating activity is due to the presence in the mucosal extract of two distinct peptides. Extracts obtained from liver, distal small intestine, pancreas or colon of animals which had undergone bowel resection did not stimulate DNA synthesis in the jejunal explants. The authors suggested this growth-stimulating factor played an important role in post resection small intestine adaptation. However, they were unable to characterize the factors other than to indicate that they were peptides of molecular weight 4500 and 1500 daltons.

In a later paper [8], these authors confirmed the presence of these two peptides in jejunal extracts of rats that had undergone a 50% proximal small bowel resection and were fed intragastrically. They were not identified in rats that had not undergone a bowel resection or had been fed intravenously following bowel resection. As in their previous studies, DNA synthesis occurred in mouse jejunal explants exposed to jejunal extract obtained from rats with previous bowel resection fed intragastrically.

Thus, the signal for small intestinal adaptation following resection and enteral nutrition may be growth stimulating peptides secreted by the remaining small intestine following luminal nutrition stimulation. These peptides are unlikely to be epidermal growth factors since they fail to stimulate growth of skin fibroblast and adenocarcinoma cells, as would be expected with epidermal growth factor. They have a specific and exclusive effect on cells of the remaining small intestine and are produced only in remaining small intestine in response to luminal nutrition post resection.

The mechanism(s) by which luminal nutrition mediates small intestinal adaptation is unclear. Nutrients may have a direct effect on the small intestinal mucosa. However, it has been postulated that luminal nutrition mediates small intestinal adaptation indirectly through the effect of pancreatic or biliary secretions on the small intestinal mucosa. Pancreatic exocrine and biliary secretion into the intestinal lumen is mediated through the effects of cholecystokinin, which is derived from endocrine cells in the gut epithelium and acetylcholine, released from parasympathetic nerve endings in the acinar tissue of the pancreas [9]. Potentially, intestinal adaptation may be mediated by factors secreted by the exocrine pancreas or by the liver through the biliary tree into the gut lumen in response to luminal nutrition.

Weser et al have attempted to determine the role of pancreaticobiliary secretions in luminal nutrition mediated small intestinal adaptation. In a 1977 study [10], they demonstrated that mucosal hyperplasia occurred in the ileum of rats following transplantation of the duodenal papilla to the ileum. Mucosal weight, DNA and protein concentration of ileal segments downstream from the site of duodenal papilla transplant increased. As well, when papilla transplant was carried out in rats that had undergone jejunectomy, the adaptive hyperplasia of the remaining small intestine was more pronounced in ileal segments adjacent to the transplanted duodenal papilla. An increase in the ratio of mucosal protein per mg DNA in ileal segments undergoing adaptation suggested adaptation in this circumstance resulted from cell enlargement (hypertrophy) more so than cell replication (hyperplasia). They theorized that ileal mucosal hyperplasia may have occurred due to a stimulatory effect of high local concentrations of biliary and pancreatic secretions in the ileum or secondary to the presence of high concentrations of undigested food in the ileum resulting from the absence of digestive enzymes in the proximal gut. They favoured the former explanation since mucosal hyperplasia in the ileum occurred in rats fed a liquid elemental diet that did not require pancreatic secretions in the proximal small intestine for digestion.

In a later study of rats fed a liquid formula diet not requiring digestive hydrolysis for absorption, Weiser et al [11] demonstrated that transplantation of the bile duct into the ileum produced ileal hyperplasia. However, hyperplasia was greater when the duodenal papilla containing both the bile and pancreatic duct were transplanted to the ileum. An increased mucosal mass in the proximal jejunum was noted in animals which had undergone transplantation of the duodenal papilla to the ileum. However, jejunal mucosal mass was not affected by bile duct diversion. They concluded that biliary and pancreatic secretions stimulate mucosal growth in the ileum and may play a direct role in ileal adaptation. As well, the mechanisms regulating ileal growth may be different than those regulating jejunal growth as the absence of biliary and pancreatic secretions stimulated proximal jejunal growth at the same time as their presence stimulated ileal growth.

In summary, the small intestine undergoes an adaptational response to proximal small intestinal resection with increased small intestinal mucosal weight, DNA and protein content and digestive enzyme activity. Proximal small intestinal resection may result in the appearance of new nutrient transporters in the remaining small intestine. However, this adaptational response following bowel resection is dependent upon the continuation of luminal nutrition. Small intestinal adaptation may be mediated by peptides released from the mucosa of the remaining small intestine or factors released in the pancreaticobiliary secretions. In the next section, the specific effect of different categories of nutrients on small intestinal adaptation will be examined.

3.3 DIETARY INFLUENCES ON ADAPTATION

Luminal nutrition is necessary to maintain intestinal integrity and allow adaptation to occur post bowel resection. However, does the specific nature of an animal's nutrition influence the small intestine's form and function? Are certain nutrients particularly important in promoting an adaptational response?

In the aforementioned study of Buts et al [4], refeeding following starvation in rats produced recovery of mucosal atrophy. However, mucosal weight recovery was significantly faster in rats fed isocaloric diets rich in lipid rather than protein hydrolysate or carbohydrates. Thus, lipids, as compared to isocaloric amounts of proteins and carbohydrates, exert a stronger trophic effect on the restoration of mucosal mass and structure. This effect was postulated to be due to more effective stimulation of enteroglucagon release by lipids than carbohydrates or proteins with resultant greater stimulation of mucosal growth.

In an in vitro study of mouse small intestine, Diamond and Karasov [12] demonstrated that feeding mice carbohydrate rich diets resulted in increased Na^+-dependent D-glucose transport in the duodenum and jejunum as compared to mice fed low carbohydrate diets. Both the maximal rate of transport (V_{max}) and the Michaelis-Menton affinity constant (K_m) of Na^+-dependent D-glucose transport were increased in mice receiving high carbohydrate diets, although the authors postulated that the increase in K_m was artifactual. They believed that this increased transport was secondary to increased D-glucose transporters per cell resulting from stimulated synthesis or decreased rate of degradation of transporters.

In a subsequent study, Ferraris and Diamond [13] studied D-glucose uptake and phlorizin binding in the small intestine of mice fed a no-carbohydrate or high-carbohydrate diet. Phlorizin is a plant glycoside which is a selective, competitive inhibitor of active D-glucose transport in the brush-border membrane of small intestine. Phlorizin has a greater affinity for the Na^+ dependent D-glucose transporter than glucose. However, unlike glucose, it is not transported into the cell. Thus, measuring radiolabelled phlorizin binding to the small intestinal mucosa allows a quantification of the number of active D-glucose transporters present in the brush border membrane. In mice fed a high carbohydrate diet, the density of glucose binding sites, as determined by phlorizin binding to the intestinal mucosa, and the V_{max} of Na^+-dependent D-glucose transport in the jejunum were 1.9 times higher than in mice fed a no carbohydrate diet. Nonetheless, significant glucose transport capacity was present in the small intestine of mice fed a no-carbohydrate diet. Phlorizin binding site density was higher in the jejunum and duodenum than ileum in mice fed a high carbohydrate diet. Thus, the small intestinal mucosa may be able to respond to specific components of luminal nutrition with adaptation representing a "tailoring" of the number of nutrient transporters to the nutrient load within the intestinal lumen. Thus, the increased number of nutrient transporters in the proximal small intestine reflects the higher concentration of nutrients in the proximal as compared to the distal small intestine.

In support of this concept, Wolffram and Scharrer [14] noted that the active transport system for amino acids in the brush border membrane adapted to increased dietary substrate levels. Increased Na^+-dependent and independent uptake of L-leucine into isolated jejunal brush border membrane vesicles (BBMV) occurred in rats fed a high protein diet as compared to rats fed a low protein diet.

In a review of the effects of different nutrients on the form and function of the intestinal tract, Thomson and Keelan [15] postulated mechanisms by which dietary manipulation could result in intestinal adaptational changes. These included alterations in the unstirred water layer, the pH microclimate of the gut, membrane fluidity, induction of solute carriers, altered distribution of transport sites along the villus, alterations of mucosal metabolism or alterations of mucosal morphology.

In the study of Ferraris and Diamond [13], more D-glucose transporters were present in the proximal than the distal small bowel. This was postulated to be secondary to the effects of higher concentrations of nutrient present in the proximal small intestine serving as a stimulus to transporter production or expression. To further study the effect of intraluminal nutrients on intestinal adaptation, Weser et al [16] examined the effects of mid-small intestine infusion of several sugars on intestinal adaptation in rats maintained on total parenteral nutrition. When compared to infusions of 0.9% normal saline, mid-gut infusions of glucose, fructose, mannose and mannitol were associated with stimulation of mucosal growth in proximity to and downstream from the site of infusion. Interestingly, 15% fructose solutions produced a greater stimulation of mucosal growth than glucose at a similar concentration. Both sugars stimulated mucosal growth in remotely distant proximal bowel segments, which, the authors suggested, may be mediated by humoral or neurovascular mechanisms. The addition of phlorizin to the infusion solution shifted the glucose-induced peak of mucosal growth caudally into more distal segments of the small bowel, but had no effect on mucosal growth induced by the other sugars. Thus, mucosal growth is stimulated by direct contact with luminal nutrients. In the case of glucose, as evidenced by the study with phlorizin, this mucosal response requires the transport of the nutrient into the cell. These studies suggest that the signal for mucosal growth initiated by luminal nutrition may have an effect locally and at a distant site.

Debnam [17] examined the effect of infusing solutions of different composition into the distal ileum of rats on the in vivo uptake of glucose or galactose by the jejunum. After a minimum 3 hour perfusion of the distal ileum with glucose or maltose, jejunal active glucose uptake was increased. Galactose, lactose and fructose perfusion of the distal ileum only minimally affected jejunal active glucose absorption. Galactose uptake in the jejunum was enhanced by ileal glucose perfusion. These responses were inhibited by the protein synthesis inhibitor cycloheximide. Postulated causes for these adaptive responses in the jejunum included a nervous reflex originating in the ileum or the release of humoral factors from the ileum, potential candidates being enteroglucagon and neurotensin.

In a subsequent study, Weser et al [18] noted that the adaptive response in rats maintained on total parenteral nutrition to midgut infusions of disaccharides was greater than that to infusions of monosaccharides. This response to disaccharides could be abolished with inhibition of disaccharidase activity. The authors proposed that the functional work load of absorbing epithelium is important in small intestinal adaptation.

In summary, small intestinal adaptation in response to luminal nutrition depends upon the nature of the luminal nutrition. Certain nutrients may be particularly effective at stimulating the secretion of mucosal growth producing hormones, such as neurotensin or enteroglucagon. The small intestine's response to increased intraluminal nutrients may be specific to that nutrient, as evidenced by the increased D-glucose transporter activity in the small intestine in response to increased intraluminal concentrations of D-glucose or non specific as evidenced by the more rapid increase in small intestinal mucosal weight in rats following starvation when fed a high lipid rather than high protein or carbohydrate isocaloric diet. Mucosal growth may be stimulated by the increased mucosal work load involved in digesting particular nutrients. Finally, nutrients may influence small intestinal adaptation as a result of modification of the intestinal microenvironment, as postulated by Thomson and Keelan [15].

3.4 HUMORAL FACTORS IN SMALL INTESTINAL ADAPTATION

Small intestinal structure and function is influenced by the nature and volume of luminal nutrition and the amount of small intestine present. The influence of the organism's internal milieu on small intestinal form and function has also been studied.

The study of the influence of humoral factors on small intestinal adaptation has been advanced by the development and application of Thiry-Vella fistulas. A Thiry-Vella fistula consists of a segment of intestine which is removed from continuity with the gut and drained externally both at its proximal and distal end. Thus, although it shares the hormonal and neurovascular milieu of the remaining gut, it has no contact with luminal nutrients or pancreaticobiliary secretions. Humoral influences on the segment of gut within the Thiry-Vella fistula can therefore be studied in isolation from the effect of luminal nutrition or pancreaticobiliary secretions. As well, hormones or nutrients can be introduced into the fistula to test the effect of these individual agents on the isolated segment of intestine. The historical background of the Thiry-Vella loop is reviewed by Keren et al [19].

Rijke et al [20] examined the effects of bypassing a segment of jejunum in rats on the cellular kinetics and maturation of the intestine in the bypassed segment. When examined at intervals of up to one year following surgery, jejunum in the Thiry-Vella fistula demonstrated a decreased number of cells per villus column with a decreased number of cells per crypt column. The total proliferative activity per crypt, as determined by scintillation counting of isolated crypts after labelling with ^{3}H-thymidine, was markedly reduced in bypassed jejunum. Deprivation of food passage and gastric, pancreatic and liver secretions thus causes a reduction in the numbers of cells per crypt, with lower cell production and a reduction in the functional villus cell compartment. This supports the critical role of direct contact of the intestinal mucosa with luminal factors in the maintenance of small intestinal integrity.

Keren et al [19] suggested a specific role for pancreaticobiliary secretions in stimulating small intestinal mucosal growth. They created Thiry-Vella loops in rabbit ileum and fed the animals orally thereafter. As early as four days post-isolation, villi in the isolated loops became shortened and blunted, with reduction of epithelial cell height and brush border thickness. Mean epithelial mitotic indices for the crypts were generally reduced. Regular perfusion of the loops with solutions containing nutrients failed to reverse these mucosal changes. The changes were reversed by reimplantation of the loops into the bowel in continuity with luminal contents. These results suggest that foodstuffs are not of primary importance in maintaining a normal mucosa. An endogenous agent, possibly in the pancreaticobiliary secretions, secreted into the upper small bowel may be critical for small bowel adaptation.

Dworkin et al [21] created proximal small intestine Thiry-Vella fistulas in two groups of rats. One group received an intragastric elemental diet containing 30% dextrose, 5% amino acids, electrolytes and vitamins while the second group received a similar diet parenterally. Gut and mucosal weights in the enterally fed rats were significantly greater in both the gut in continuity and the fistula as compared with those rats fed parenterally. These differences were also seen in analysis of the protein and DNA content of the intestine of each group. Although contact with intraluminal factors was important in determining the adaptive response of the small intestine, this study showed that hormonal or neurovascular stimuli must also be important in small intestinal adaptation since the mucosal growth was greater in the Thiry-Vella fistula of rats fed enterally than in those fed parenterally.

Thus, Thiry-Vella fistulas have helped establish that intestinal growth and adaptation occurs both in response to local and systemic factors. Studies have been carried out to determine the identity of these systemic factors.

Schwartz and Storozuk [22] established in a rat model that infusion of gastrin into an isolated mid small intestine Thiry-Vella loop results in increased intestinal absorption of labelled galactose and glycine by the loop as compared to uptake from Thiry-Vella loops in control animals. These results suggested that gastrin is capable of enhancing intestinal substrate absorption.

Elias and Dowling [23] examined the effect of lactation in rats on small intestine in isolated proximal small intestine Thiry-Vella loops. As well, they examined the effects of duodenal transposition to mid-point of small bowel, with exclusion of bile and pancreatic secretions to the jejunum, on the intestinal structure and function in both the ileum and jejunum. They compared results in these two models with intestinal structure and function in control groups of lactating and non-lactating rats. Villus height and total mucosal thickness as well as intestinal glucose absorption were examined. Lactation caused an increase in total mucosal thickness with increased villus height as well as crypt depth to a similar degree in all three groups of lactating rats. Mucosal hypoplasia and diminished mucosal thickness were not seen in Thiry-Vella bypassed segments of jejunum in lactating rats. There was enhanced glucose absorption per unit length of intestine in the lactating groups as compared to non-lactating controls. Enhancement was more pronounced in the lactating ileum, with the mean value being more than 2 1/2 times greater than in the non-lactating ileum. Thus, pancreaticobiliary secretions and luminal nutrition are not important in stimulating small intestine mucosal growth in lactation. The stimulus for mucosal growth in lactating mammals could be a hormone released either from the gut or from adjacent organs. Glucagon was postulated to be a possible responsible agent.

In a second study of lactating rats, Yang et al [24] correlated ornithine decarboxylase levels with small intestinal adaptive changes accompanying lactation. Inhibition of ornithine decarboxylase activity by administration of α-difluoromethylornithine suppressed intestinal adaptation, with a decrease in the adaptive increase in mucosal weight and thickness, particularly in crypt depth. Thus, ornithine decarboxylase may have an important role in cell proliferation in the crypt proliferative compartment in lactation. Increased ornithine decarboxylase activity might be the trigger for mucosal cell proliferation in certain adaptive conditions of the small bowel.

Similar to Elias and Dowling [23], Jacob et al [25] suggested that enteroglucagon had a role in intestinal adaptation. At a symposium on mechanisms of intestinal adaptation, they reported studies in which intestinal tissue and fasting plasma enteroglucagon levels were measured in three models of small intestine adaptation; small bowel resection, lactation and hypothermia induced hyperphagia. Increased levels of enteroglucagon immunoreactivity were present in fasting plasma and small intestinal tissue of animals that developed adaptive mucosal hyperplasia in each of these models suggesting that enteroglucagon was trophic to the small intestine.

Other hormones have been examined for their role in small intestine mucosal growth and adaptation. Hughes et al [26] demonstrated in dogs that small intestinal mucosal hypoplasia occurring during total parenteral nutrition could be prevented in both proximal and distal intestinal segments by intravenous therapy with cholecystokinin and secretin. It was unclear, however, whether this trophic effect was secondary to a direct effect of the hormones on the intestine or an indirect effect mediated by hormonal stimulation of pancreaticobiliary secretions. Similarly, Morin and Ling [27] demonstrated stimulation of small intestine growth by intravenously administered pentagastrin in rats fed parenterally following a 50% proximal small bowel resection. Growth stimulation only occurred in proximal small intestinal segments and the authors therefore suggested that mucosal growth was the result of gastrin stimulated gastric or exocrine pancreatic secretion.

Weser et al [28] demonstrated in rats that the intravenous administration of octapeptide-cholecystokinin stimulated mucosal growth with growth being enhanced by the addition of secretin. Glucagon did not stimulate growth. These authors also suggested that mucosal growth may have resulted from the indirect effect of secretin and octapeptide-cholecystokinin stimulated pancreaticobiliary secretions rather than as a direct effect of the hormones on the small intestinal mucosa.

In summary, the study of humoral influences on small intestinal growth and adaptation has been advanced by the use of Thiry-Vella fistulas. These have allowed researchers to study intestinal growth in isolation from the effects of intraluminal factors. Studies using Thiry-Vella fistulas have confirmed the role of luminal nutrition and pancreaticobiliary secretions in small intestinal mucosal growth and adaptation. Studies have suggested possible roles for enteroglucagon, ornithine decarboxylase, gastrin and perhaps secretin and octapeptide-cholecystokinin in mucosal growth. However, the effects of many of these hormones may not be a direct trophic effect on the small intestine mucosa but rather an indirect effect due to hormonal stimulation of pancreaticobiliary secretion.

3.5 SMALL INTESTINAL ADAPTATION IN DIABETES MELLITUS

Small intestinal form and functional capacity respond to changes in the organism's nutritional and hormonal milieu. The small intestine also responds to changes in the body's internal environment related to underlying illness.

DM has become a major focus of study, because of the clinical importance of this disorder as well as possibly because this disease has proven to be an interesting model of adaptation. Characterized by inadequate pancreatic secretion of insulin to meet the demands of the peripheral tissues, DM may occur as a result of an absolute insulin deficiency, as seen in Type I DM, or as a result of peripheral insulin resistance coupled with inadequate production of insulin by the pancreas, as seen in Type II DM. The result in either case is increased serum glucose levels associated with relatively reduced serum insulin levels.

DM can be experimentally induced by the intravenous injection of streptozotocin, which is toxic to the insulin producing islet cells of the pancreas. Using this model, the acute and chronic effects of DM can be studied. The adaptational response of the small intestine to hyperglycaemia and altered levels of insulin can be examined and the intestinal response to correction of hyperglycaemia and hypoinsulinemia determined.

The effects of acute hyperglycaemia on small intestinal nutrient transport have been studied. In 1976, Csaky and Fisher [29] demonstrated that mucosal to serosal transport of glucose and galactose by an everted jejunal sac in rats was increased if the animals received a 4 hour intravenous glucose infusion rendering them hyperglycemic prior to sacrifice. Fructose transport was not increased in similar circumstances. However, fructose transport could be increased when rats were pretreated for 4 or more hours with intravenous fructose. Increased sugar transport was inhibited by phloretin, which affects glucose transport at the basolateral membrane but not by phlorizin, which affects transport at the brush border membrane. Increased sugar transport did not occur in rats treated with cycloheximide, which inhibits protein synthesis, during the intravenous sugar infusion. The authors concluded that hyperglycaemia enhances small intestinal sugar uptake as a result of the formation of new transport carriers with resultant increased sugar uptake. Inhibition of this increased uptake by phloretin and not phlorizin suggested that this acute adaptational change occurs at the level of the basolateral mewmbrane (BLM) and not the brush border membrane (BBM) of the enterocyte. As well, this adaptive response is substrate specific.

This conclusion was supported by the studies of Maenz and Cheeseman [30]. They demonstrated, in jejunal BLM vesicles prepared from acutely hyperglycemic rats, that the maximal velocity of Na^+ independent facilitated D-glucose transport was 78% greater than that seen in normoglycemic rats. Interestingly, increased D-glucose uptake was not seen in BLM prepared from the ileum. Neither Maenz and Cheeseman [30] or, in a similar study, Karasov and Debnam [31] noted any change in the dynamics of D-glucose transport across the BBM under conditions of acute hyperglycaemia. Thus, rapid adaptation of intestinal D-glucose transport to changes in the serum glucose level are mediated by changes at the BLM and not the BBM. The signal for this adaptational change is unclear. However, Karasov and Debnam [31] postulated that hormonal or neurological signals might be involved.

The small intestine's response to chronic DM has been studied. In rats with streptozotocin-induced DM, Granneman and Stricker [32] demonstrated increased intestinal mass, glucose absorption and disaccharidase activity in the small intestine as compared to control non diabetic animals. These changes occurred both in rats fed a low carbohydrate high fat and a high carbohydrate low fat diet, but were more pronounced in rats fed high carbohydrate diets. Rats fed high carbohydrate diets became hyperphagic while rats fed a low carbohydrate diet ate normal amounts of food. Increased gastric emptying of carbohydrates but not lipids was also noted in the diabetic rats. They concluded that DM, independent of hyperphagia, increases intestinal digestive enzyme activity and glucose absorption. Hyperphagia may be a behavioral adjustment to accelerated transit of nutrients through the gastrointestinal tract.

In a study of rats with streptozotocin-induced DM, Fedorak et al [33] noted enhanced 3-0 methylglucose absorption in the ileum and jejunum, with enhanced L-alanine absorption in the ileum. Maximal velocity of uptake (V_{max}) was increased, while the Michaelis constant (K_m) was not significantly changed. Treatment of diabetic rats with insulin completely eliminated these adaptive changes. The authors hypothesized that these changes occurred as a result of recruitment of additional BBM glucose carriers in the diabetic state.

Later studies by Fedorak et al confirmed this hypothesis. In a 1989 study [34], using autoradiography techniques, they determined the binding of [^{3}H]phlorizin to the ileal mucosa of diabetic and non diabetic control rats. Binding was 7.2-fold greater in diabetic rats than non-diabetic rats. This binding occurred in the mid-villus region as well as at the villus tips, whereas in control rats, it was confined to the villus tips. The authors concluded that, in rats, D-glucose uptake normally is limited to villus tip enterocytes. However, in diabetic rats, recruitment of D-glucose carriers in previously non-glucose transporting mid-villus enterocytes occurs as these enterocytes adapt to a role in glucose transport.

Fedorak et al [35] subsequently examined the response of the jejunum and ileum to streptozotocin induced DM in the rat in both acutely (14 days following induction of DM) and chronically (60 days following induction of DM). Using an in vitro [^{3}H] phlorizin autoradiography technique, they established that, in the jejunum, with chronic DM, there is an increase in the density of upper villus glucose carriers with no acute affect while, in the ileum, the number of upper villus glucose transporters increases with recruitment of mid villus carriers both in the acute and chronic diabetic state. Thus, the adaptational response to DM differs in the ileum as compared to the jejunum and consists of an increase in the absolute number of D-glucose transporters with induction of D-glucose transport at a more proximal level of the crypt-villus axis.

Satoh et al [36] examined the time sequence of changes in nutrient uptake in BBMV isolated from the small intestine of guinea pigs with streptozotocin-induced DM. Within 3 days of treatment with streptozotocin, increased intestinal Na^+ dependent L-amino acid transport and decreased Na^+ dependent D-glucose transport were noted. However, by 10 days following induction, the uptake of amino acids had normalized while glucose uptake was twice as high as in normal guinea pigs. These changes were attributed to changes in V_{max} values without associated changes in K_m values.

Dudeja et al [37] studied D-glucose transport along the crypt villus axis in the jejunum of streptozotocin-induced diabetic rats. After 10-14 days of being diabetic, Na^+-dependent D-glucose uptake in BBMV harvested from diabetic rats at the mid-villus and lower-villus levels was significantly higher than that found in non-diabetic rats at the same levels. Kinetic analysis of these values showed that two glucose transporters were present along the crypt villus axis, a low affinity, high capacity transporter in mid-villus and villus tip cells, and a high affinity, low capacity transporter in the brush border membrane of crypt cells. The K_m recorded at these levels was the same in diabetic as compared with non-diabetic rats, with significantly increased V_{max} present in the mid-villus and lower villus BBMV. The authors concluded that increased glucose uptake at mid and lower villus level in the diabetic rat was secondary to recruitment of more glucose carriers at that level.

Debnam and Ebrahim [38] studied the uptake of L-valine in streptozotocin induced diabetic rats as compared to control rats. Rats were fed radiolabelled L-valine and autoradiographs of the jejunal villi 7 days and 30-40 days following the induction of DM were made. In the control rat, L-valine uptake was limited to the distal 20-23% of the villus whereas in the acutely and chronically diabetic rat, uptake occurred in the upper 42-45% of the villus. As well, villus height was increased in diabetic rats with an increased enterocyte lifespan. They suggested that, in DM, there is an earlier maturation of enterocyte absorptive functions resulting in the villus containing a higher proportion of mature enterocytes capable of nutrient transport. A similar response was noted in diabetic rats fed equivalent amount of food as control rats and diabetic animals fed ad libitum suggesting that hyperphagia is not an important factor in adaptation in diabetes mellitus.

Thomson et al [39] established that the enhanced nutrient uptake encountered in DM can be modified by dietary manipulation. Enhanced uptake of glucose and galactose was noted in the jejunum and ileum of diabetic rats fed a standard rat chow or a diet enriched in saturated fatty acids. However, uptake in diabetic rats fed a diet enriched in polyunsaturated fatty acids was similar to that of control non diabetic rats.

Thus, using different techniques, these authors have demonstrated that small bowel adaptation in DM involves the formation of more small intestine nutrient transport carriers. However, the signal for this increase in the number of nutrient carriers in the small intestinal villus is unclear. Could the reduced insulin levels and not the elevated serum glucose levels seen in DM be the stimulus for adaptational change in the small intestine?

Research has been advanced to answer this question. In the aforementioned study of Fedorak et al [33], treatment of diabetic rats with insulin completely prevented the increased uptake of 3-0 methyl glucose noted in the jejunum and ileum of diabetic rats. Similarly, Thomson and Rajotte [40] noted that the increased jejunal uptake of galactose and glucose found in diabetic rats as compared to non diabetic control rats was reduced by the daily injection of insulin or by islet cell transplantation. These authors were unable to clarify the molecular events responsible for increased nutrient uptake in the untreated diabetic animals.

Fujii et al [41] have suggested that activity of the Na^+-dependent D-glucose transporter in the BBM may be physiologically controlled by insulin. They studied Na^+ dependent D-glucose uptake in BBMV prepared from normal control rats and streptozotocin induced diabetic rats which were either starved, treated with insulin or allowed to eat ad libitum but not treated with insulin. Although similar blood glucose levels were seen in the starved and insulin treated diabetic rats, glucose uptake was increased above that of the control non diabetic rats only in the starved diabetic. Glucose uptake in vesicles prepared from the diabetic rats fed ad libitum was similar to that of the diabetic starved rats and was 1.8 fold higher than the uptake of glucose by either the control or the insulin treated diabetic animals. Increased binding of phlorizin to the BBMV of both the diabetic fed ad libitum and starved rats was noted. These findings suggest that intestinal glucose transporter activity is controlled by insulin with the signal for transporter production being reduced amounts or absence of insulin. This study did not implicate a role for hyperphagia in diabetes induced small intestinal adaptation.

In summary, the small intestinal mucosa responds with adaptational changes to both acute hyperglycaemia and chronic hyperglycaemia as seen in DM. The acute response to hyperglycaemia occurs at the level of the BLM and may involve the formation of more transporters at this level. The chronic response to hyperglycaemia and hypoinsulinemia is seen at the BBM and consists of an increase in the number of both glucose and amino acid transporters. Transporters also appear at more proximal levels of the crypt-villus axis than in the non diabetic state. The signals initiating these changes are as yet unclear. However, the adaptational change does not depend on the serum glucose levels per se but rather is influenced either directly or indirectly by serum insulin levels. DM associated hyperphagia with resultant increased intraluminal nutrient levels does not appear to play a role in DM related small intestinal adaptation.

3.6 ADAPTATIONAL RESPONSE TO EXTRAINTESTINAL EVENTS

Survival of the organism is dependent on the small intestine's ability to absorb nutrients. Nutrient absorption must be able to adapt to adversity. The response of the small intestine to bowel resection, dietary changes, DM and lactation has been examined. Radiation exposure and senescence are two further stimuli to small bowel adaptation that have been studied. An understanding of the small intestine's response to these stressors has important clinical applications. As the population in Western society is rapidly aging, physicians need to better understand nutrient utilization and requirements in the elderly. As well, the spectre of nuclear war and nuclear accidents such as the Chernobyl nuclear accident remain all too real possibilities for the future such that the small intestine's response to radiation and methods to improve gut function following radiation exposure may have importance in future clinical medicine. Models have been established to examine the small intestine's adaptational responses to these situations.

Cheeseman et al [42] studied the effects of abdominal irradiation on intestinal transport in isolated rat enterocytes. Three days following total body irradiation with a sub-lethal dose of 600 rads, small bowel villus height and mucosal surface area were markedly reduced. By 14 days following radiation, the gross morphology of the small bowel returned to normal. Active uptake of D-glucose, which was significantly enhanced at 3 days following radiation, had returned to preradiation therapy levels by 14 days following irradiation. A reduced cell yield was noted within three days of irradiation which similarly returned to that of control animals within 14 days of radiation therapy. Thus, immediately following irradiation, the small intestine compensates for a reduced cell population by increasing the transport capacity of individual enterocytes. With recovery of the cell population, absorptive capacity normalizes again. L-leucine uptake changes did not parallel those of glucose uptake. L-leucine uptake was unaffected at 3 days following irradiation and depressed at 14 days. Adaptational small intestinal nutrient uptake changes following radiation thus appear to be non uniform and substrate specific. Radiation induced small intestinal adaptation and possible therapies for radiation induced enteropathies have recently been reviewed [43,44].

With aging, changes occur in an organisms metabolism and nutrient requirements. Doubek and Armbrecht [45] compared glucose uptake by everted intestinal sacs in young, adult and old rats. Na^+-dependent D-glucose uptake was significantly higher in the young (2-3 month old) rats as compared to the older (12-14 and 24 month old) rats. When glucose transport was studied using brush border membrane vesicles, this decreased uptake was further noted, with no change in the K_m but a decline in the V_{max} with age. Thus, as animals age, the number and/or activity of Na^+-dependent glucose carriers may decline.

Freeman and Quamme [46] reached a similar conclusion in a study of Na^+ dependent glucose transport in jejunoileal BBMV harvested from rats between 3 and 156 days in age. Two D-glucose transporters were identified in the small intestine of rats less than 8 weeks old: a high capacity and low affinity system located in the jejunum and a low capacity and high affinity system locate throughout the small intestine. By contrast, in rats over 8 weeks of age, both systems were found along the length of the jejunum and ileum. There was a greater capacity for Na^+ dependent glucose transport present in young animals as compared to mature rats. Thus, aging may involve a reduction in the number of active nutrient transporters in the small intestine. The signals for these changes have yet to be determined. The spectrum of changes in the function of the gastrointestinal tract associated with aging has been further reviewed by Thomson and Keelan [47].

3.7 CONCLUSION

Small intestinal adaptation is a process of functional and morphological changes of the enterocytes of the crypt villus axis in response to signals coming from the intestinal lumen, the cytosol of the cell or the bloodstream. In cases of nutrients actively transported at the level of the BBM, regulation of the number of responsible carriers may be the main mechanism of adaptation. Intraluminal factors may be of major importance, with dietary substrates potentially regulating their intestinal transporters. As reviewed by Ferraris and Diamond [48], increasing levels of intraluminal nutritional substrates result in up-regulation of transporters while increased intraluminal levels of trace minerals and some vitamins result in down regulation of their transporters. However, the role of intraluminal nutrition in many circumstances of adaptation may be overshadowed by systemic or other intraluminal factors. The signals for these changes are unclear, but may be secondary to release of hormones trophic to the small intestine or nerve stimulation. Either direct or indirect trophic effects have been described for enteroglucagon, octapeptide-cholecystokinin, gastrin and insulin. Pancreaticobiliary secretions may contain factors capable of stimulating intestinal growth and development. Future studies may allow us to manipulate the adaptive response in disease states such as DM, radiation exposure or following bowel resection in order to optimize patient nutrition.

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CHAPTER 4

GLUCOSE UPTAKE BY THE SMALL INTESTINE

4.1 INTRODUCTION

Approximately 300 gm of carbohydrates are ingested daily by the average adult in Western society; about 45% of the total calories consumed. Of these carbohydrates, 60% consists of polysaccharides, 30% consists of the disaccharide, sucrose, and 10% consists of the disaccharide, lactose.

Carbohydrate metabolism begins upon oral contact with food. Secondary to the effects of salivary amylase, pancreatic amylase and the disaccharidases: sucrase/isomaltase complex, glucoamylase/ maltose complex, and lactase/phlorizin complex, disaccharides and complex carbohydrates are metabolized to produce monosaccharides, mainly glucose, for absorption by the small intestinal absorptive epithelial cells (enterocytes). Greater than 95% of all ingested non fibre carbohydrate is absorbed by the small intestine in humans [1].

Since glucose constitutes the major product of carbohydrate breakdown, it is critical to understand how glucose is absorbed from the small intestinal lumen. In this chapter, the historical background of the study of glucose uptake by the small intestine will be presented. As well, studies on the nature of the glucose uptake process and the characteristics of the glucose transporter will be reviewed. Results of studies analyzing glucose uptake along the crypt-villus axis (CVA) and along the small intestinal axis from the duodenum to the ileum will be reviewed.

4.2 HISTORICAL PERSPECTIVE

Prior to 1949, investigation of nutrient uptake by the small intestine was inhibited by the lack of experimental preparations able to maintain small intestinal viability outside the organism. In that year, Fisher and Parsons [2] described an in vitro preparation of small intestine which enabled them to study nutrient transport in a surviving intestine preparation. In their preparation, the lumen of an intestinal segment was cannulated both proximally and distally and perfused with an O_2 saturated CO_2-bicarbonate buffered solution in an anaesthetized rat. After intestinal perfusion was accomplished, the animal was sacrificed with subsequent removal of the intestinal segment and suspension of the perfused segment in a heated bath of O_2 saturated CO_2-bicarbonate buffered solution. The concentrations of nutrients in the perfusing (mucosal surface) and bathing (serosal) solutions could be varied and measured over time to establish fluxes of nutrients across the intestinal wall.

In a subsequent paper [3], they used the above described preparation to measure glucose movement across the small intestinal wall. After one hour of exposure to serosal and mucosal solutions initially containing equal concentrations of glucose, greater concentrations of glucose were noted in the solution perfusing the serosal surface of the intestine, suggesting that translocation of glucose across the intestinal wall from mucosal to serosal surface had occurred. Translocation of glucose was greater in segments harvested from the proximal jejunum as compared to segments harvested from the ileum with a linear increase in glucose uptake as the distance from the ileo cecal valve increased. Utilization of glucose by the intestinal segments was also determined and did not vary along the length of the small intestine. Absorption of glucose was decreased when phlorizin was introduced to both serosal and mucosal bathing mediums, to an average of 26% of the control rate. When glucose was placed only in the serosal surface bath, the disappearance rate of glucose from this solution was equal to the intestinal segment's calculated utilization rate with no significant appearance of glucose in the mucosal surface solution.

Thus, rat small intestine could translocate glucose from the mucosal to serosal side of the intestine against a concentration gradient implying the presence of active glucose transport. This process was inhibited by phlorizin and was more active in proximal than distal segments of the small intestine with a linear gradient of translocation activity occurring from proximal to distal small intestine.

In a subsequent study using a similar technique, Fisher and Parsons [4] examined the effect of varying the concentrations of glucose in the mucosal and serosal bathing solutions on the rate of glucose absorption across the intestinal wall. Varying the glucose concentration in the serosal bathing solution did not alter the rate of disappearance of glucose from the mucosal bathing solution. However, the rate of absorption of glucose from the mucosal perfusing solution varied with the initial glucose concentration in the mucosal perfusing solution. When the reciprocal of the initial glucose concentration in the mucosal perfusing solution was plotted against the reciprocal of the average absorption rates, a linear relationship existed which could be represented empirically by an equation of the Michaelis and Menten type. Thus, glucose absorption from the small intestine had a limiting velocity (V_{max}) and a characteristic half-saturation transport was not influenced by the concentration of sodium in the serosal solution. No measurable glucose transport occurred when sodium was present in the serosal bathing solution but not in the mucosal bathing solution. The inhibition of glucose transport by the absence of Na^+ ions was rapidly reversed by the reintroduction of Na^+ ions to the mucosal bathing solution.

In a subsequent in situ study, Csaky and Zollicoffer [7] demonstrated the importance of Na^+ ions in the absorption of glucose from the small intestine in rats. In this study, the absorption of glucose from an isolated segment of perfused jejunum was determined. Active transport of glucose from this segment only occurred in the presence of Na^+ ions in the mucosal perfusing solution, thus demonstrating the critical role of Na^+ in the absorption of glucose in vivo in mammals.

Thus, by 1960, it was established that glucose transport from the small intestinal lumen is an active process occurring only under aerobic conditions in the presence of Na^+ ions within the intestinal lumen. Active transport adheres to Michaelis-Menten mechanics with a maximum rate of glucose absorption and a half saturation concentration (K_m). Uptake varies along the length of the intestine and is greater in proximal segments than distal segments of the small intestine. Glucose uptake is inhibited by phlorizin and seems to be accelerated in the presence of K^+ ions.

Theories were subsequently developed to explain the mechanism by which sodium influenced glucose movement across the small intestinal wall. In 1961, Csaky [8] demonstrated that the active transport of 3-0 methylglucose, L-tyrosine, phenylalanine or uracil from a solution bathing the mucosal surface of isolated frog small intestine to the serosal surface of the intestinal segment is significantly reduced in the absence of sodium ions. Since the stimulatory effect of Na^+ ions was not exclusive for glucose transport, Csaky postulated that active transport of nutrients occurred through the use of a two component biological pump consisting of a carrier and a system which converts chemical to osmotic energy. He postulated that sodium was primarily necessary for the second component of the pump, namely, the one which converted chemical to osmotic energy. He believed that the initial component, the carrier, was specific for each substance transported. However, the second component was non specific and applicable to the transport of different substrates.

A year later, Crane [9] developed an alternative model of sodium-mediated glucose transport into the cell. In Crane's model, the active transport process is located at the enterocyte brush border pole. A mobile carrier-sugar complex is present in the brush border which complexes with Na^+ ion(s). Once complexed with the Na^+ ion(s), it is able to traverse the brush border into the cell cytoplasm where it releases the glucose molecule and Na^+ ion(s). An active transport system hence returns the Na^+ ion(s) to the intestinal lumen while the glucose remains in the cytoplasm, unable to be transported out of the cell in the absence of sodium. Thus, glucose transport into the cell occurs in the presence of a sodium gradient since, were the concentration of sodium in the cell to be equal to that outside the cell, glucose would be transported from the cell interior to exterior at the same rate as it was transported in the opposite direction. He further postulated that entry of glucose into the cell could occur in anaerobic conditions in the presence of sodium in the luminal medium, in an energy independent sodium dependent fashion, as long as a sodium gradient was present.

Which of these theories was correct? In 1963, Csaky [10] again demonstrated a critical role of sodium in D-glucose transport across toad and rat small intestinal mucosa. The positive effect of sodium on glucose and 3-0 methylglucose transport did not occur until one hour after sodium was introduced into the mucosal perfusing media. He theorized that sodium's effect on glucose transport only occurs after it enters the cell, which requires time. As well, when the concentration of glucose in the intestinal lumen was greater than that present in the blood in an in situ rat preparation, the uptake of glucose or 3-0 methylglucose from the intestinal lumen was the same whether or not sodium was present in the luminal solution. Uptake of glucose or 3-0 methylglucose from the small intestine in these circumstances of high substrate concentrations was inhibited by phlorizin implying that a sugar carrier was involved in glucose transport which did not depend on sodium for its functioning. He presented this data as further support of the concept of glucose transport into the cell being via a two component system.

In a study using an everted intestine model in 1965, Crane et al [11] studied uptake by hamster small intestine of the glucose analogue 6-deoxyglucose under circumstances of varying sodium concentrations. When the sodium concentration in the lumenal medium was lowered towards zero, the maximum rate of 6-deoxyglucose transport remained constant while the K_m increased suggesting that carrier affinity for glucose increased with increasing Na^+ ion luminal concentration. They postulated that three asymmetries existed which influenced sodium dependent glucose transport from the small intestinal lumen; an inward and downhill gradient of sodium, an outward and downhill potassium gradient and a gradient of substrate carrier affinity. Each of these asymmetries depended on the energy dependent translocation of sodium out of the cell. They postulated a mobile carrier within the brush border membrane (BBM) which possessed binding sites for glucose and monovalent cation(s) that migrated freely between interfaces of the cell membrane. Under circumstances of high glucose concentration, the carrier could transport glucose in the absence of sodium, as observed by Csaky [10].

Which of the above theories was correct could not be determined in the absence of a method to evaluate glucose transport at the level of the BBM in isolation from the cell. Existing in vitro preparations for the study of nutrient uptake by the small intestine suffered from the complicating effects of cellular metabolism and the additional barriers to transport in the subepithelial, muscularis and serosal layers of the small intestine.

In 1968, Forstner et al [12] described a technique for isolating the BBM of small intestinal cells with low levels of subcellular contamination. Mucosal scrapings from segments of rat small intestine were homogenized and added to solutions of EDTA and NaCl. Subcellular components were subsequently removed through a combination of homogenate filtration through glass-wool and differential centrifugation. The technique's validity was confirmed by electron microscopy showing that the BBM fraction consisted almost entirely of membranous structures having the same cross sectional width and structure characteristics of the intact BBM. As well, enrichment in the BBM fraction of hydrolytic enzymes associated with the BBM and decreased concentrations of DNA and RNA as compared to those found in the intestinal homogenate were noted, again confirming the validity of their technique.

In 1973, Hopfer et al [13] isolated BBM from rat jejunum and ileum and examined their transport capabilities using a modification of the above technique. Hopfer's group included a step in which $MgSO_4$ was added to the solution of homogenized small intestine causing a cross linking of intra-cellular organelles and basal-lateral membrane to the divalent Mg^{2+} ion to form complexes which precipitated from solution. These subcellular organelles and membranes could be removed during subsequent centrifugation steps. The resultant solution was enriched in BBM, as demonstrated by a 31-fold increase in invertase (INV) activity in the vesicle solution as compared to INV activity in the homogenate of intestinal scrapings. Electron microscopy studies confirmed that the resultant BBM fragments formed vesicles. Using these brush border membrane vesicle (BBMV) preparations, the uptake of both D-glucose and L-glucose in the presence of a sodium gradient and in the presence of other cations was studied.

A glucose carrier system was identified in the BBMV which was specific to D-glucose, with transport of D-glucose exceeding that of D-galactose and L-glucose. Active vesicular uptake of D-glucose only occurred when Na^+ ions were present in the incubation medium and was inhibited by phlorizin. Measured over time, D-glucose uptake was maximal in the first 5 minutes of incubation, subsequently decreasing to a point of zero net D-glucose uptake. When expressed graphically, these results demonstrated an "overshoot" of glucose uptake in the presence of sodium over time. D-glucose uptake was not affected by the presence of other ions such as Li^+ or K^+ in the incubation medium. However, it decreased as the gradient of Na^+ ions from the incubation medium to the intravesicular space decreased. During incubation of vesicles in hypertonic solution, glucose uptake was inhibited suggesting that uptake of glucose was into an intravesicular space and not binding to the vesicular wall.

Thus, a specific sodium gradient dependent transport system for D-glucose was demonstrated in the BBM of the small intestine. Since these studies were conducted in the absence of an energy source, they provided clear evidence for a sodium-dependent facilitated diffusion system for glucose in the brush border membrane. D-glucose transport depended on the presence of a gradient of sodium concentration across the walls of the BBM since, as the gradient approached zero, net flux of glucose across the membrane also approached zero. Crane's theory was thereby confirmed and Csaky's theory regarding the existence of a two-component glucose transport system disproved.

In summary, early studies of glucose uptake by the small intestine using oxygenated intestinal segments and in vivo preparations demonstrated that glucose uptake is an active process that depends upon the presence of a sodium gradient from intestinal lumen to intestinal wall, adheres to Michaelis-Menten mechanics, does not occur in anaerobic conditions and is inhibited by phlorizin. Subsequent studies using purified BBMV demonstrated that glucose uptake occurs as a result of a sodium-dependent facilitated diffusion system in which both glucose and sodium are transported by a D-glucose specific transporter in the BBM. Active glucose transport into the cell only occurs in the presence of a sodium gradient across the BBM which is maintained by an energy dependent process of sodium transport from the interior to exterior compartment of the cell.

4.3 GLUCOSE TRANSPORT INTO THE ENTEROCYTE

There are two barriers to glucose transport from the intestinal lumen into the absorptive cell; the unstirred water layer (UWL), and the BBM.

The UWL, which is adjacent to all biological membranes, constitutes a barrier between the BBM and the intestinal lumen. It consists of a series of water lamellae extending outward from the BBM, each progressively more stirred, until the layers blend imperceptibly with the bulk water phase. Movement across this layer is determined by diffusional forces with resistance determined by three parameters; 1) the effective thickness of the UWL which is higher in the crypt as compared to the villus tip and higher in the jejunum that ileum [14]; 2) the surface area of the UWL; and 3) the free diffusion coefficient of the probe molecule across this layer.

The second major barrier to glucose transport into the cell is the BBM. The development of a method for the isolation of vesiculated BBM, with preservation of membrane enzymes and transporters, marked a turning point in the study of nutrient transport in the small intestine. Subsequent isolation techniques have centred upon the technique pioneered by Hopfer et al [13]; precipitation of non-BBM cellular material from homogenates of small intestinal mucosa by the divalent ions Ca^{2+} or Mg^{2+} followed by differential centrifugation to separate the BBM from subcellular material. Using this technique, transport across the BBM could be studied in isolation from the remainder of the small intestine. Transport of nutrients in different incubation medias could be studied with variations in the electrolyte and nutrient concentrations in the intravesicular and incubation media easily achievable. Transport could be studied at different positions both along the CVA and along the intestine from duodenum to ileum. Models could be developed for the study of nutrient uptake in disease states, such as diabetes mellitus (DM), starvation, lactation, following radiation exposure or post bowel resection. The influence of diet and senescence on uptake characteristics of the small intestine could be examined. Finally, the very structure of the transporters in the brush border membrane could be analyzed.

The advantages of BBMV over other biological preparations of small intestine for the study of nutrient and electrolyte flux across the BBM have been elucidated by Semenza et al [15]. Advantages of the use of vesicles in the study of nutrient uptake by the small intestinal BBM include: (i) they are free or nearly so of subcellular metabolic enzymes and of cell organelles and are similarly free of substrates other than those added by the experimenter; (ii) it is possible to control, at least to a large extent, the composition of the incubation media on both sides of the membrane including the osmolarity and pH, (iii) vesicles are not subject to the restrictions imposed by a surviving tissue (iv) they can be expected to have a negligible UWL as compared to intact tissue, (v) they allow a determination in principle of the accumulation of "free" substrates and thus estimation of the energy conversion yields: (vi) they are a sizable step in the purification of membrane transport agencies (vii) they allow one to test the effect of permeant versus impermeant reagents on some functions of the transporter ; (viii) solute movements observed to occur across the membrane are not distorted by movements across paracellular pathways; (ix) BBMV are the most stable biological preparation from the small intestine in which transport can be studied: they are easy to prepare; (x) they require small volumes and minute amounts of substrates; (xi) as incubations can be as short as a few seconds, it is possible to follow the absorption of unstable substrates (xii) with the use of vesicles, far fewer animals need be sacrificed to provide tissue.

In this section, studies examining the nature of glucose transport across the BBM will be reviewed. As well, studies of glucose uptake along the intestine and along the CVA will be analyzed.

In a rat small intestine model, Murer and Hopfer [16] demonstrated that sodium-gradient dependent D-glucose transport into intestinal BBMV is dependent upon the generation of an electrical potential gradient across the vesicle wall. Sodium-coupled D-glucose translocation across the BBM is electrogenic, with the positive charge associated with the Na^+ ion not compensated by the co-movement of an anion, or the counter movement of a cation via the glucose carrier across the vesicular wall. They theorized that an electrogenic (Na^+-K^+)- ATPase in the lateral basal layer coupled with high Na^+ conductance of the tight junction between epithelial cells would enable Na^+ ions to return to the intestinal lumen and then move into the cell via the brush border membrane glucose-sodium carrier.

Using a cyanine dye which fluoresces in the presence of a positive membrane potential, Steiger et al [17] confirmed Murer and Hopfer's [16] findings. They demonstrated in rat small intestine BBMV, that, in the presence of D-glucose and extravesicular sodium, the fluorescence of the dye increased indicating the formation of a transient inside positive membrane potential. This did not occur in the presence of L-glucose or in the absence of Na^+ ion in the incubation medium. Preloading of vesicles with sodium and D-glucose and incubating them in a buffer containing sodium but not D-glucose led to decreased fluorescence suggesting that the efflux of D-glucose resulted in a negative membrane potential.

Initial studies of sodium gradient dependent glucose uptake in BBMV were carried out using rat small intestine. Subsequent studies using tissue from other species have confirmed the presence of sodium gradient dependent glucose transport in the small intestine BBM in these species as well. They have also helped characterized glucose transport both along the duodenal-jejunal-ileal axis and along the CVA.

In 1950, Parsons and Fisher [3] established that a gradient of glucose uptake occurred along the small intestine, with greater glucose uptake in the proximal small intestine than in the distal small intestine. Using human small intestinal BBMV isolated from organ donor intestine, Harig et al [1] demonstrated that sodium gradient dependent D-glucose transport occurred along the entire length of the small intestine. They also confirmed Fisher and Parsons' findings, demonstrating that uptake was greatest in the distal jejunum with uptake less in the proximal jejunum and lowest in the distal ileum. Using Eadie-Hofstee analysis, the maximum rate of glucose absorption (V_{max}) and a half saturation concentration (K_m) by the BBM in both the proximal jejunum and terminal ileum were determined. At least two glucose transport systems were present in the proximal jejunum and one system in the terminal ileum; a high affinity system with a relatively low capacity for glucose transport and a low affinity, high capacity glucose carrier in the jejunum with a low affinity, high capacity glucose carrier present in the distal ileum. They postulated that the high capacity, low affinity carrier was suited to its position in the proximal jejunum where it could transport high concentrations of glucose found in the proximal small intestine postprandially while the high affinity carrier in the distal ileum would be well suited for glucose salvage in the distal ileum with its low post prandial concentrations of glucose. In a later study of BBMV prepared from human donor duodenum, Harig et al [18] demonstrated that sodium gradient dependent glucose transport occurred in the duodenum as well as the jejunum and ileum. However, uptake in the duodenum was less than that noted in the jejunum.

Bluett et al [19] demonstrated a similar gradient of glucose uptake along the small intestine in a study utilizing BBMV prepared from human small intestine harvested at surgery. They confirmed that glucose uptake in the jejunum and proximal ileum was sodium gradient dependent and electrogenic. However, unlike Harig et al [1], they did not demonstrate sodium gradient dependent glucose uptake in the terminal ileum. Harig et al [1] explained these results differences by suggesting that BBMV prepared from surgical specimens may not be optimal for study due to prolonged and uncontrolled ischemia of the mucosa prior to harvesting.

In a study of BBMV purified from jejunoileal segments of rat small intestine, Freeman and Quamme [20] established the presence of two sodium-dependent glucose carriers in the intestine of young rats under 7-8 weeks of age; one located in the jejunum characterized by high capacity and low affinity, and a second located throughout the jejunum and ileum and characterized by a low capacity and high affinity. In rats 12-13 weeks of age and older, the low affinity transporter was found along the length of jejunum and ileum. Thus, more than one transporter was found in the small intestine with development of transporters being age dependent.

Keljo et al [21] studied sodium gradient dependent D-glucose uptake by jejunal BBMV prepared from piglet jejunum. Uptake was studied in vesicles isolated from healthy piglets and piglets with acute viral diarrhea. Activity of lactase and INV were significantly less in the mucosal homogenates and BBMV prepared from piglets with viral diarrhea as compared to healthy piglets. Sodium dependent D-glucose uptake was present in both groups of animals. However, Edye-Hofstee analysis of D-glucose uptake suggested that two carrier populations were present in vesicles prepared form the normal population; a high affinity and a low affinity D-glucose transporter while only a single low-affinity transporter was present in vesicles prepared from diseased animals. Since, in acute viral diarrhea, small intestinal enterocyte maturation is impaired and the mucosa predominantly consists of less differentiated crypt cells, the presence of two transporters in the healthy and not diseased piglets may be consistent with a model in which a single low affinity D-glucose transporter is present in the crypt cells with a second transporter appearing during maturation of the cells along the CVA.

Kaunitz and Wright [22] studied sodium D-glucose cotransport in bovine jejunal BBMV. They established the presence of two sodium gradient dependent glucose transport systems; a major, high capacity system with low glucose affinity which was detectable in the presence of D-glucose concentrations in the incubation medium greater than 0.25 mM and a minor high affinity low capacity system detectable at D-glucose concentrations below 0.25 mM.

In a study using jejunal guinea pig BBMV, Brot-Laroche et al [23] demonstrated the presence of two distinct glucose transport systems. One system, termed the "low temperature sensitive system" was obligatorily dependent on the presence of a sodium gradient to function. It had a high affinity and low capacity for D-glucose transport and was unaffected by temperature changes between 25 and 35·C. The second system, termed the "high temperature sensitive system" was most active at the temperature 35·C with negligible glucose affinity as the temperature approached 25·C. It was hypothesized that this was a low cation specific system which could accept lithium and potassium as well as sodium and had a low affinity but high capacity for glucose transport. The existence of this transporter was postulated after D-glucose uptake by BBMV in the absence of sodium was noted at a rate much higher than would have been anticipated if movement across the membrane was purely passive.

More recently, study of sodium gradient dependent D-glucose transport in human fetal jejunum BBMV by Malo [24] demonstrated the presence of two distinct sodium dependent D-glucose transporters. Kinetic studies using an Eadie-Hofstee plot of sodium dependent D-glucose as a function of increasing concentration of D-glucose in the incubation medium demonstrated the presence of a high affinity, low capacity transporter and a second low affinity, high capacity system. In the presence of 3-0 methyl glucose in the incubating medium, Eadie-Hofstee analysis demonstrated the presence of only the low affinity high capacity transporter. These results suggested that the high affinity, low capacity transporter was inhibited by 3-0 methyl glucose while the second carrier was unaffected. Thus, two glucose transporters are present in the human fetal jejunum. Results from this study, however, do not predict similar findings in the postpartum human.

Using an in vitro preparation of rabbit jejunum, Thomson et al [25] similarly demonstrated that more than one glucose carrier was present in the small intestine. They studied uptake by the tissue over a determined time period of radiolabelled glucose, galactose and 3-0 methylglucose when the hexoses were added alone or in combinations of two or more to the mucosal side of the tissue. Kinetic analysis of uptake data demonstrated that there was not a single common carrier for all three sugars. Graphic analysis and non-linear regression studies suggested there were at least three hexose carriers in the jejunum which had different affinities and capacities for the transport of each hexose. Alternatively, they postulated that only one hexose carrier was present which behaves differently as it matures along the CVA. They were unable however to prove or disprove either of these theories.

Thus, studies of small intestinal glucose transport suggested that there were at least two sodium dependent D-glucose transporters present in the small intestinal BBM. Indeed, potentially there were more than two with carrier distribution varying along the length of the intestine.

Thomson et al [25] and Keljo et al [21] have suggested that the development of more than one sodium dependent D-glucose transporter might occur as a function of enterocyte maturation. Several studies have subsequently examined glucose uptake along the CVA.

Sugar and amino acid absorption by everted sacs of hamster jejunum was studied using autoradiography techniques by Kinter and Wilson [26]. Nutrient uptake was determined by measurement of radioactive activity on autoradiographs of histological segments of small intestine. Maximal activity occurred at the villus tip, with activity being almost undetectable at the bases near the mouths of the crypts. However, with prolonged incubations, activity was noted in the crypts. Addition of phlorizin to the lumenal solution reduced sugar related activity in the cells, predominantly at the brush border pole of enterocytes. The presence of most autoradiographic activity in the upper villus suggested that sugars were mainly absorbed by cells of the upper villus. Therefore, glucose transport capacity may increase as a function of cell maturation as enterocytes advance along the CVA. Nonetheless, the authors suggested, increased activity noted in the cells of the upper villus may not have indicated increased uptake of nutrients by these cells but rather a greater rate of exit of nutrients from cells in the crypt.

Autoradiographic studies by Fedorak et al [27] similarly suggested that glucose uptake occurs mainly in the upper villus segment. They examined the distribution of sodium coupled D-glucose carriers in the BBM along the CVA of rat ileum using [^{3}H]phlorizin autoradiography. Phlorizin binds to the glucose carrier in the brush border membrane without being transported into the enterocyte. Thus, radiolabelling phlorizin and incubating it with small intestinal segments allows the density of glucose carriers along the axis to be determined on autoradiographic study of histological sections of the intestine. Uptake of [^{3}H]phlorizin was limited to the upper portions of the villus with patchy binding to the side wall of the villus and, on many sections, absent binding below the level of the upper villus. These studies suggested that functioning glucose transporters were limited to the upper villus and villus tip segments of the small intestine. Since glucose uptake requires glucose carriers, sodium gradient dependent glucose uptake must principally occur in the upper villus.

Using micropuncture measurements of membrane potentials, Stewart and Turnberg [28] studied uptake along the CVA. Cells of the villus and crypt in an in vitro preparation of rat ileum were impaled by microelectrodes and BBM potentials and fractional resistance determined. A decline in membrane potential suggested that an electrogenic active transport process was occurring across the membrane. The introduction of glucose to the mucosal side of the preparation, in the presence of sodium, resulted in a decrease of membrane potential, consistently in the mid-third and upper third of the villus, occasionally at the villus base, but not at the level of the crypt or the villus crypt junction. The presence of phlorizin, which blocked active glucose transport, completely eliminated this depolarization, confirming that depolarization was secondary to glucose transport across the BBM. These results confirmed that glucose uptake occurred principally in the upper villus. Not all cells of the upper villus demonstrated a change in membrane potential on exposure to glucose suggesting that not all cells in the upper villus are involved with glucose transport.

Thus, autoradiographic and membrane potential recording techniques suggest that glucose uptake occurs predominantly in the upper villus segments of the CVA. Potentially, glucose uptake is a function of cell maturation. Glucose uptake by the BBM along the CVA has been studied using BBMV preparations in order to further characterize glucose uptake along the CVA.

In 1973, Weiser [29] established a technique of isolating enterocytes sequentially from along the CVA. Segments of intestine were harvested from rat small intestine. Enterocytes were subsequently separated from the underlying villus by agitation of the intestinal segments with harvesting of successive fractions of cells. In theory, the first enterocytes to be agitated off the villus were the most distal villus tip enterocytes with enterocytes subsequently "falling off" the villus from progressively more proximal along the CVA. The origin of the cell fractions from along the CVA was confirmed by analysis of cellular enzyme profile of the cell fractions. These studies demonstrated higher alkaline phosphatase (ALP) and INV activity in initially harvested (upper villus cells) fractions as compared to final isolated fractions (crypt cells) with increasing levels of thymidine kinase (TK) activity in the final isolated fractions as compared to initially isolated fractions, confirming the validity of this harvesting method.

Thus, a method was established that allowed the isolation of cells from identifiable levels of the CVA. Cell fraction position along the CVA could be determined by analysis of the cellular enzyme profile. Techniques were subsequently developed for the isolation of BBMV from these fractions in order to study the nature of glucose uptake by the BBM along the CVA.

Freeman et al [30] sequentially isolated cells from along the CVA of proximal rat small intestine. As in Weiser's study [29], they demonstrated a gradient of ALP and TK activity along the CVA. BBMV were isolated from villus and crypt segments of the harvested cells and sodium gradient dependent D-glucose transport was measured in each fraction. Sodium gradient dependent D-glucose uptake was detected in BBMV prepared from both the villus and crypt cells and was greater in the villus fraction than in the crypt fraction. Using kinetic analysis of the resultant data, two transporters were defined in the villus fraction: a low affinity-high capacity transporter with V_{max} 4.28 nmol·mg protein $^{-1}$·min^{-1} and K_m 499 μM and a high affinity low capacity transporter with V_{max} 0.62 nmol·mg protein^{-1}·min^{-1} and K_m 9.47 μM. In the crypt segment, only one transporter was detected; a high affinity-low capacity transporter with V_{max} 3.00 nmol·mg protein^{-1}·min^{-1} and K_m 68.3 μM. They postulated that the rate of glucose transport is differentiation dependent and relates to the degree of enterocyte development.

Meddings et al [31] isolated three enterocyte fractions from along the CVA of New Zealand white rabbit small intestine; one from the villus tip, one from the mid-villus segment and one from the crypt. They similarly validated their cell fractionation technique by demonstrating a gradient of BBM enzyme activity along the CVA. BBMV were prepared from each fraction and D-glucose transport rates measured with varying D-glucose concentrations during 5 second incubation periods. D-glucose uptake was lowest in crypt BBMV and highest in mid-villus and villus tip derived vesicles. Mid-villus and villus tip vesicles demonstrated a low affinity and high capacity for D-glucose transport with V_{max} 47.75 nmol·mg protein^{-1}·min^{-1} and K_m 200.4 μM in vesicles isolated from villus tip cells and V_{max} 40.03 nmol·mg protein^{-1}·min^{-1} and K_m 242.4 μM in vesicles isolated from mid-villus cells. Vesicles isolated from the crypt cells demonstrated a high affinity and low capacity for glucose transport with V_{max} 7.68 nmol·mg protein^{-1}·min^{-1} and K_m 114.3 μM.

Meddings et al [31] acknowledged that there may be different glucose transporters along the CVA. However, by exposing vesicles to benzyl alcohol, they were able to fluidize villus tip brush BBMV to levels of fluidity seen in the crypt BBMV. Kinetic analysis of glucose uptake by the benzyl alcohol treated villus tip cell derived vesicles demonstrated that the transporter's glucose affinity and maximal glucose transport capacity were now similar to those noted in crypt cell derived vesicles. Thus, they postulated, the same glucose transporter was present at all levels of the CVA which expresses itself differently in different lipid environments. In the rabbit, the BBM is more rigid (decreased fluidity) at the villus tip than in the crypt. They suggested that glucose uptake was dependent on the glucose carrier being able to make conformational changes following uptake of sodium. These changes could occur more easily in the more fluid crypt BBM. Thus, the carrier would have a greater affinity for glucose in the crypt as compared to in the more rigid villus tip brush border membrane. However, fluidity changes along the CVA do not explain differences in the V_{max} of glucose transport in the crypt versus the upper villus. Possibly, the lower V_{max} in the crypt derived vesicles resulted from fewer glucose transporters being present at that level of the CVA.

Dudeja et al [32] studied D-glucose uptake in BBMV prepared from Lewis rat proximal small intestine. D-glucose uptake in fractions derived from villus tip, mid villus and lower villus enterocytes was determined and it was demonstrated that uptake was maximal in vesicles derived from villus tip cells. Kinetic parameters of sodium gradient dependent D-glucose uptake demonstrated low affinity and high capacity for glucose transport in vesicles derived from villus tip and mid villus cells with V_{max} 904 pmol·mg protein^{-1}·4sec^{-1} and K_m 121 μM calculated for vesicles isolated from villus tip cells and V_{max} 1296 pmol·mg protein^{-1}·4sec^{-1} and K_m 164 μM for vesicles isolated from mid-villus cells. Vesicles isolated from crypt cells demonstrated a high affinity and low capacity for glucose transport with V_{max} 498 pmol·mg protein^{-1}·4sec^{-1} and K_m 44 μM.

Fluidity of BBMV along the CVA was determined. In the Lewis rat, fluidity decreased as cells matured along the CVA. This was opposite to what Meddings at al [31] had noted in the New Zealand white rabbit. Despite this difference, a gradient of glucose uptake along the CVA was recorded that, similar to the findings of Meddings et al [31], showed increased uptake in the villus tip and mid-villus segments of the CVA as compared to uptake in the crypt segment. This data implied that there was no consistent relation between fluidity and glucose uptake by the BBM. Differences in uptake by the crypt BBM as compared to the mid-villus and villus tip membranes could be accounted for by the presence in the immature crypt cells of a high affinity, low capacity glucose carrier with the appearance, during cell maturation, of a low affinity, high capacity carrier in the upper villus. The relative fluidity of the BBM along the CVA was not a generalized property in all species but was species specific.

Using monoclonal antibodies to the sodium-glucose cotransporter, Haase et al [33] determined the distribution of the cotransporter along the CVA and in different segments of the small intestine. Histological sections of small intestine were prepared and exposed to the monoclonal antibody. Antibody binding was visualized after incubation of the sections with fluorescein-isothiocyanate labelled anti-mouse IgM antibodies. Subsequent staining procedures allowed visualization on light microscopy and electron microscopy of the sites of localization of the monoclonal antibodies and, therefore, the locations of the sodium glucose cotransporter.

Studies using this technique confirmed that cotransporter density is higher in the jejunum than in the duodenum or ileum, with density of glucose cotransporters estimated to be 30% lower in the ileum and 40% lower in the duodenum than in the jejunum. Immunostaining was confined exclusively to the mucosal membranes with complete absence of staining in the basolateral membrane and the cell bodies of the enterocytes. The cotransporter was distributed homogenously per surface area of BBM along the CVA from within the crypts to the villus tips. However, since mature villus cells have a larger area of BBM per cell than immature crypt cells, as the cells advance along the CVA, they contain progressively more sodium glucose cotransporter in their BBM.

In summary, sodium gradient dependent glucose transport across the BBM is electrogenic. Uptake varies along the length of the intestine with maximal uptake occurring in the distal jejunum and less uptake occurring in the proximal jejunum, duodenum and ileum. Autoradiographic, membrane potential measurement and BBMV glucose uptake studies have demonstrated that uptake of glucose predominantly occurs in the upper villus and villus tip segments of the CVA. Potentially, more than one sodium gradient dependent glucose transporter is present in the small intestine. Transporters can be differentiated by their differing affinities for glucose and other hexoses. Studies using monoclonal antibodies raised against the sodium glucose cotransporter have confirmed that the cotransporter is more abundant in the jejunum than in the duodenum or ileum. These studies have also demonstrated that cotransporter density per unit brush border membrane is equivalent along the entire CVA. However, since upper villus cells have more BBM surface area than is present in cells of the lower villus and crypt, they contain more cotransporter per cell than is present in crypt and lower villus cells.

Thus, transcellular transport of glucose with facilitative sodium gradient dependent movement of glucose across the brush border membrane has emerged as the principal theory explaining glucose transport from the intestinal lumen. However, a theory has emerged suggesting that sodium dependent transcellular glucose transport is not the only major mechanism of glucose transport from the intestinal lumen. This alternative mechanism will be reviewed in the next section.

4.4 PARACELLULAR GLUCOSE UPTAKE

In three studies published in 1987, Pappenheimer et al [34, 35, 36] suggested that significant movement of glucose or amino acids from the small intestinal lumen occurred as a result of solvent drag through paracellular channels. In this model, sodium gradient dependent transcellular uptake of glucose from the intestinal lumen activates contraction of the perijunctional actomyosin ring in the apical region of epithelial cells. This results in the opening of tight junctions thereby permitting the transit of luminal nutrients in bulk by a process of solvent drag into the subepithelial tissue. Thus, the role of sodium coupled transport is to transport nutrients and sodium into the intercellular lateral spaces below the occluding junction, provide the osmotic flow for absorption of fluid and trigger contraction of cytoskeletal proteins.

In theory, this mechanism of nutrient absorption is most active in circumstances of high nutrient concentration in the gut lumen, as occurs in the duodenum and proximal jejunum. In the distal small intestine where nutrient concentrations are low, active transport was postulated to be the major mode of nutrient absorption.

Evidence was presented in support of this model. Madara and Pappenheimer [36] demonstrated on electron microscopy study of isolated segments of hamster small intestine that, in the absence of glucose in the intestinal lumen, the intercellular spaces were collapsed with closely apposed lateral membranes. However, upon exposure to glucose, tight junctions between enterocytes developed structural deformities in the form of dilatations. They postulated that this resulted in occluding junctions becoming more permeable and lateral spaces expanding to provide optimal conditions for transport of luminal nutrients in bulk by solvent drag between intestine cells.

In a second study, Pappenheimer and Reiss (34) studied nutrient and fluid uptake in an in vivo rat preparation. Rats were anaesthetized and segments of intestine were cannulated. Solutions containing varied amounts of solutes were perfused through the intestinal segments and fluid absorption and clearance of solutes determined. In the presence of glucose, uptake from the lumen of the inert hydrophillic solutes inulin, polyethylene glycol, creatinine and phenolsulfonpthalein was increased. The presence of glucose in the intestinal lumen also resulted in increased fluid absorption with fluid absorption in the absence of luminal glucose being only 50% of that in its presence. They postulated that the increased permeability of the small intestine to these molecules and fluid occurred as a result of increased width of intercellular channels induced by active transport of glucose.

In a third study, Pappenheimer [35] demonstrated that the addition of glucose to the lumen side of isolated segments of rat or hamster intestine reduced the transepithelial impedance. It was postulated that the addition of glucose to the luminal contents had induced an opening of the intercellular junctions with subsequent solute transport by solvent drag and decreased transepithelial impedance.

In a later study, Atisook et al [37] demonstrated, in isolated segments of hamster epithelium, that activation of the sodium glucose cotransporter is the triggering event in increasing tight junction permeability. Using electron microscopy, they demonstrated that dilatations of tight junctions in villus absorptive cells occurred in the presence of mucosal glucose but could be prevented both by replacement of mucosal glucose with choline or by addition of phlorizin to the mucosal solution. In an in vivo preparation similar to that used by Pappenheimer and Reiss [34], they also noted increased creatinine clearance from the intestinal lumen in the presence of glucose in the intestinal lumen. This increased creatinine clearance was prevented by the addition of phlorizin to the luminal perfusing solution. Using transcellular flux of mannitol to estimate paracellular glucose flux, they calculated that, at physiologically realistic in vivo luminal glucose concentrations, as much as 30% of glucose absorption occurs via paracellular absorption.

In summary, glucose uptake from the intestinal lumen may occur as a result of anatomically definable changes in tight junctions permeability between enterocyte absorptive cells. Subsequent movement of fluid across these more permeable tight junctions results in movement of nutrients into the subepithelial space by a process of solute drag. The role of sodium gradient dependent glucose transport is critical to this process as the active transport of glucose and sodium into the intercellular lateral spaces below the occluding junction provides the osmotic flow for absorption of fluid and triggers contraction of cytoskeletal proteins required for the subsequent process of solvent drag. Thus, paracellular transport of glucose constitutes a major route of glucose transport from the intestinal lumen. However, it appears to be of most importance during periods of high intraluminal glucose concentration. When the intraluminal glucose concentration is low, sodium gradient dependent glucose transport is the principle route of glucose absorption. Thus, sodium gradient glucose transport across the brush border membrane remains critical to glucose absorption from the small intestinal lumen.

4.5 MOLECULAR BIOLOGY OF THE BRUSH BORDER GLUCOSE TRANSPORTER

The discovery of a sodium glucose cotransporter in the BBM raised many questions regarding the nature of nutrient transport into the enterocyte. How many molecules of sodium are transported per glucose molecule? How close is the sodium carrier on the transporter to the glucose carrier? By what mechanism is glucose moved out of the cell after being transported into the cell by the cotransporter? These questions have been addressed by researchers and will be reviewed in this section.

Initial studies examined the nature of the interaction between sodium and glucose in the glucose transporter. Using rabbit small intestinal BBMV, Kaunitz et al [38] studied glucose uptake in the presence of an increasing concentration of sodium. They established that at least two and potentially as many as 4

Na$^+$ ions were involved in glucose transport with a 2:1 or greater sodium to glucose coupling ratio in rabbit small intestinal BBM. These results were confirmed by Freeman and Quamme [20] in a study of glucose uptake by BBMV prepared from rat jejunoileal mucosa.

Thus, two or more Na$^+$ ions are coupled to glucose during transport across the BBM. However, what is the physical relationship between the Na$^+$ ion and the glucose molecule on the sodium glucose cotransporter? How does the attachment of one of these moieties affect the attachment of the other to the carrier?

Peerce and Wright [39], using rabbit small intestinal BBM, studied the kinetics and conformational changes of the glucose carrier during the transport of glucose and sodium. BBMV were studied using fluorescence and it was established that sodium binds to the glucose transporter and, by doing so, causes a conformational change in the transporter which increases its affinity for glucose.

In a subsequent paper, Peerce and Wright [40] used covalent fluorescent probes, which bound to the sodium and glucose binding sites on the rabbit intestinal BBM sodium-glucose cotransporter, to measure the distance between the two binding sites. They calculated this distance as between 30 and 40 Å and estimated the molecular weight of the sodium-glucose cotransporter as approximately 75000 Dalton. They suggested that, in a polypeptide of this molecular weight, this was a substantial distance. The sodium induced conformational changes in the carrier would have to be extensive to affect affinity at such distances.

Thus, by 1986, researchers had determined the basic nature of the sodium glucose cotransporter. The cotransporter is a 75000 Dalton molecular weight polypeptide able to bind glucose and 2 or more Na$^+$ ions with a distance of 30-40 Å between glucose and sodium binding sites. Binding of sodium induces conformational changes in the cotransporter resulting in increased transporter affinity for glucose. Subsequently, glucose and sodium are transported to the inner surface of the BBM and released. This release likely involves further conformational changes in the carrier. Further research studies were focused on determining the molecular composition of the carrier.

In 1987, Hediger et al [41] reported the successful cloning of the rabbit intestinal sodium-glucose cotransporter. The transporter consists of 662 amino-acid residues, with a relative molecular mass of

73,080 Dalton. Two highly charged hydrophillic segments were identified which might be involved with glucose binding. The glucose transport kinetics of both the cloned glucose transporter and the glucose transporter in rabbit BBM were later studied [42]. These studies demonstrated that, although qualitative and quantitative differences existed between the two transport systems, the cloned transporter was indeed the same glucose sodium cotransporter as that found in the rabbit small intestine BBM.

In subsequent studies from the same laboratory [43], it was determined that the gene encoding for the sodium glucose cotransporter in humans is located on chromosome 22. In this paper, the authors expressed the hope that this further characterization of the sodium/glucose cotransporter would be helpful in understanding the autosomal recessive disorder "glucose/galactose malabsorption."

In summary, the molecular biology of the BBM sodium-glucose cotransporter has been studied. The cotransporter is a polypeptide with a molecular weight of 73080 dalton. It is able to bind glucose and sodium in a ratio of at least two Na^+ ions per molecule of glucose with a distance between the Na^+ ions and the glucose molecules of 30 to 40 Å. Binding of sodium induces conformational changes in the cotransporter which allow glucose to subsequently bind with greater affinity. The cotransporter has been successfully cloned in both the rabbit and human. In the human, the cotransporter gene is present on chromosome 22. Localization of this gene's location may be helpful in understanding the autosomal recessive disorder "glucose/ galactose malabsorption."

Thus, much has been learned regarding glucose transport from the intestinal lumen across the BBM. However, before intracellularly transported glucose can be utilized by the body, it must be transported from the enterocyte, across the basolateral membrane (BLM). In the next section, the glucose transport from within the enterocyte across the BLM to the subepithelial space will be reviewed.

4.6 GLUCOSE TRANSPORT ACROSS THE BASOLATERAL MEMBRANE

Basolateral membrane vesicles (BLMV) can be prepared using nitrogen cavitation techniques and density gradient centrifugation. Wright et al [44] prepared BLMV from rat duodenum, mid-jejunum and ileum. They confirmed their preparation were enriched in BLM by demonstrating enrichment of (Na^+-K^+)-ATPase activity with reduced activities of INV, as a marker of BBM contamination, and succinate dehydrogenase, as a marker of mitochondrial contamination as compared to activities in the initial intestinal mucosal homogenate. They subsequently examined the transport of sugars into the BLMV.

Their studies demonstrated that transport of glucose across the BLM was stereospecific with greater affinity being demonstrated for D-glucose than for L-glucose or galactose. Carrier mediated transport of glucose across the BLM was not influenced by the presence of sodium. Uptake of D-glucose exhibited saturation kinetics, with elimination of carrier mediated uptake in the presence of $HgCl_2$ or phloretin. Significant inhibition was provided by phlorizin and cytochalasin B. Cytochalasin B, a fungal metabolite, inhibits energy independent facilitated D-glucose diffusion in mammalian cells, such as erythrocytes. Thus, D-glucose transport across the BLM, in contrast to the glucose transport at the BBM, is a facilitated diffusion system with transport completely independent of sodium.

In a recent study by Thorens et al [45], the liver facilitated diffusion glucose transporter was cloned from rat liver cDNA libraries and antibodies prepared in rabbits against the COOH terminus of the transporter. Histological sections of rat small intestine were incubated with these antibodies. The distribution of the facilitated diffusion glucose transporter could hence be determined along the CVA and in different segments of the small intestine by measurement of immunofluorescence in sections following incubation with fluoresceinated goat anti-rabbit immunoglobulin antibody.

These studies demonstrated that the facilitated glucose transporter in the small intestine is the same transporter polypeptide, with tissue specific posttranslational modifications, as found in the liver, kidney and pancreatic islet β cells. Immunofluorescence studies of rat small intestine demonstrated that it was restricted to the BLM of enterocytes. In the jejunum, ileum and duodenum, immunofluorescence was not seen in cells in the crypts nor in the cells at the base of the villi, only being seen in BLM of enterocytes of the mid and upper villi. It was therefore suggested that this transporter is a marker for differentiation of the intestinal epithelial cells.

In summary, glucose transport from the cell occurs across the BLM via a sodium independent facilitated diffusion carrier. Transport across the BLM may be studied using vesicles prepared through differential centrifugation and nitrogen cavitation techniques. This carrier is similar to the facilitated diffusion carrier seen in the liver and kidneys. It is stereospecific for D-glucose. Immunofluorescence studies have suggested that it is mainly distributed in the more mature villus cells than in the immature crypt and basilar villus cells.

4.7 CONCLUSION

The process of glucose transport from the small intestinal lumen has been studied intensively in the last 45 years. Transport of glucose into the cell occurs via a sodium gradient dependent electrogenic glucose-sodium cotransporter in the BBM. This process does not directly require energy. However, energy is required to maintain a sodium gradient across the membrane. Studies of glucose transport across the BBM have been advanced by the use of techniques allowing the isolation of the BBM in vesicles with preservation of intact membrane transport systems and BBM enzymes. Sodium gradient dependent glucose transport is most active in the jejunum with lesser activity in the ileum and duodenum. Studies using different avenues of approach suggest that glucose transport is a function of cell maturation and, therefore, is more active in absorptive cells of the upper villus than in absorptive cells of the crypt and lower villus.

The relationship between glucose and sodium in the transporter and the structure of the sodium glucose cotransporter in the BBM have been determined. Binding of sodium by the cotransporter induces a conformational change in the transporter that increases the affinity of the carrier for glucose. Studies have also examined the role of glucose in promoting the movement of fluid and nutrients from the intestinal lumen to the subepithelial space through paracellular pathways. These studies suggest that sodium gradient dependent glucose transport by the cell may be the initiating factor for movement through paracellular pathways via solvent drag of fluid, glucose and other nutrients.

The transporter in the BLM has been studied. This is a sodium independent glucose transporter that is similar to transporters found in the liver, kidney and pancreas. It is distributed predominantly along the upper and mid villus being absent in cells of the crypt and lower villus.

In viewing glucose uptake from the gut lumen, one can see this as a multi step complex process, with initial intraluminal hydrolysis of carbohydrate followed by diffusion of disaccharides and monosaccharides across the UWL, sodium-dependent transport of glucose across the BBM, transit across the cell, and finally exit through the BLM into the circulation. In the section on small intestinal adaptation, the adaptational response of small intestinal glucose uptake in different adaptational circumstances will be reviewed. Sodium dependent transport across the BBM may be modified by a change in the number of glucose carriers, as is the postulated model for adaptation in DM, or, potentially, a change in the characteristics of the carrier itself. Dietary factors may influence glucose carrier activity as may exposure.

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CHAPTER 5

LIPID ABSORPTION

5.1 INTRODUCTION

The average adult in Western society consumes between 60 and 100 grams of fat daily, constituting approximately 50% of the daily caloric intake. Most dietary fat consists of triglycerides, consisting of three molecules of fatty acid and one molecule of glycerol, with the remainder consisting of phospholipids and cholesterol esters. The majority of triglycerides contain saturated or unsaturated fatty acids with chain lengths greater than 14 carbon atoms. Dietary fat also contains essential fat-soluble vitamins A, D, E and K, as well as fatty acids essential for the synthesis of prostaglandins, leukotrienes and thromboxanes.

Because fat is hydrophobic, unlike carbohydrates and proteins which are hydrophillic, it is unable to freely diffuse from the intestinal lumen to the brush border membrane (BBM). Therefore, special mechanism are required for the absorption and utilization of dietary fat.

The overall process of fat digestion and absorption can be divided into seven phases. These are: 1) emulsification of fats and dispersion into small particles; 2) enzymatic hydrolysis of fatty acid esters; 3) dispersion of digestion products into a form suitable for absorption; 4) diffusion across the unstirred water layer (UWL); 5) absorption across the enterocyte BBM; 6) intracellular metabolism and packaging of lipids into lipoproteins; 7) transport from epithelial cells to the lamina propria [1].

In this chapter, the absorption of lipids from the intestinal lumen across the UWL and BBM and finally the mechanisms of lipid transport within the enterocyte will be discussed. Studies examining the passive and active uptake of lipid by the small intestine will be presented. As well, fatty acid binding proteins (FABP) and their role in lipid uptake will be discussed. Lipid movement across the UWL and the role of bile acids in facilitating this process will be analyzed. Finally, the role of dietary lipid in the modulation of nutrient uptake by the small intestine will be reviewed. In the first section of this chapter, the intraluminal events leading up to the presentation of lipids to the BBM for absorption will be reviewed.

5.2 INTRALUMINAL LIPID DIGESTION

The digestion of dietary fat begins in the stomach as a result of the strong shearing forces produced by gastric muscle contractions. These forces result in emulsification of dietary fats to the size of 0.5 μm lipid droplets. While in the stomach, triglycerides are exposed to lingual lipase. Secreted by serous glands in the back of the tongue, this enzyme is active in the acidic gastric environment and hydrolyses dietary triglycerides to diglycerides and protonated fatty acids.

With gastric emptying and the presentation of lipid emulsions to the duodenum, cholecystokinin is released from duodenal mucosal cells. Under the effects of this hormone, the gallbladder contracts and bile salts are released into the intestinal lumen. As well, the pancreas is stimulated to secrete both bicarbonate and digestive enzymes into the intestinal lumen. Peristaltic and segmental contractions in the duodenum supply mechanical energy that reduce the size of the lipid droplets further.

Subsequent hydrolysis of dietary fat occurs secondary to the effects of the pancreatic lipase. Pancreatic lipase is secreted by the pancreas in its active form with an pH optimum of 8.0. It is capable of hydrolysing the 1 and 3 ester bonds of the triglyceride molecule thereby producing 2-monoglyceride and free fatty acid. It is only able to cleave triglycerides at the interface between the oil and aqueous phase. Its activity is inhibited by bile acids as well as the presence of protein and phospholipids in the intestinal lumen; possibly as a result of inhibition of the lipophilic bonding of pancreatic lipase to triglycerides at the aqueous-oil interface. It differs from lingual lipase in that lingual lipase activity, unlike pancreatic lipase activity, is not appreciably influenced by physiological concentrations of phospholipids and has a broader pH activity range [2].

The activity of pancreatic lipase is enhanced by the presence in the intestinal lumen of pancreatic colipase. This coenzyme is secreted by the pancreas in equimolar amounts to lipase as a procoenzyme requiring intraluminal hydrolysis by trypsin for activation. Once activated, colipase binds to the ester bond region of the triglyceride molecule. Lipase then binds to colipase and hydrolysis of the triglyceride by lipase occurs, even in the presence of bile salts. Thus, lipase is able to hydrolyse triglycerides, even in the presence of bile salts or phospholipids. In addition to binding to lipase, colipase is capable of binding to the bile salt micelle. Shiau [3] has theorized that, as a result of colipase binding to bile salt micelles in close proximity to the oil-water interface, rapid solubilization of lipolytic products into micelles occurs. The resultant rapid removal of lipolytic products from the interface subsequently facilitates further lipolysis by lipase.

Following intraluminal lipolysis, products of lipid digestion include free fatty acids, 2-monoglycerides and cholesterol. These molecules have low water solubility. Thus, only small amounts are dissolved in the intraluminal bulk phase and absorption by enterocytes across the UWL would be minimal. Lipid absorption is facilitated by the formation of mixed micelles of bile salts and lipid.

Bile acids constitute the end products of cholesterol metabolism. In humans, the "primary" bile salts synthesized by the liver include conjugates of cholic and chenodeoxycholic acid with "secondary" bile salts, deoxycholic and lithocholic acid, produced in the intestine by bacterial dehydoxylation of primary bile salts. Each bile salt molecule contains a fat soluble sterol nucleus and a polar, water soluble hydroxyl and amino groups. The polar hydroxyl and amino groups are oriented to the same side of the bile acid molecule in the aqueous phase of the intestinal lumen leading to a strongly hydrophillic as well as hydrophobic pole. At low concentrations in aqueous solutions, bile acids exist as monomers. However, above a certain concentration termed the critical micellar concentration, they spontaneously form negatively charged aggregates or micelles of between 20 and 50 molecules which are negatively charged and between 30 and 100 Å in size. Bile salt micelles are spherical, with the polar hydroxyl and amino groups facing outward towards the outer aqueous phase and the nonpolar steroid hydrocarbon nuclei facing inward forming a nonpolar interior.

The critical micellar concentration varies with the species of bile salt, the pH and the temperature. The presence of a negative charge at the polar end of the molecule allows the entire micelle to become dissolved in the aqueous phase. Because of its nonpolar hydrophobic inner pole, the micelle is able to dissolve the monoglyceride and free fatty acid products of triglyceride metabolism. The micelle thus formed is termed a mixed micelle. In the next section the role of the mixed micelle in facilitating the absorption of lipid by the BBM will be reviewed.

In summary, lipid metabolism begins upon exposure of dietary fat to the acidic environment of the stomach. Under the influence of the shearing forces generated by gastric smooth muscle contractions, fat is emulsified. As well, under the influence of lingual lipase, metabolism of triglycerides to free fatty acids and monoglycerides is initiated. In the small intestine, pancreatic lipase is able to continue the hydrolysis of triglycerides, under the influence of colipase. Once hydrolysed to monoglycerides and free fatty acids, the products of lipid metabolism, as well as free cholesterol, are incorporated into mixed micelles for subsequent absorption across the UWL and BBM into the enterocyte

5.3 LIPID MOVEMENT ACROSS THE UNSTIRRED WATER LAYER

Before an intraluminal solute can enter the cytosol of the enterocyte across the BBM, it must first traverse the UWL. The UWL consists of a series of water lamellae extending outward from the BBM, each progressively more stirred, until the layers blend imperceptively with the bulk phase. Immediately adjacent to the BBM a region of static fluid is present in which thermal convection or density gradients do not cause any significant mixing of the solution. Movement across the UWL is via simple diffusion in which the rate of movement of a solute molecule is determined by the functional thickness of the UWL (d), the aqueous diffusion constant of the molecule (D) and the concentration gradient between the bulk water phase (C_1) and the underlying BBM (C_2). The rate of flux of a solute across the UWL (J) can thus be expressed by the equation $J=(C_1-C_2)D/d$.

Postulated mechanisms by which bile acid micelles facilitate lipid movement across the UWL have been reviewed by Thomson and Dietschy [4]. Three models of lipid uptake from the mixed bile salt-lipid micelle have been proposed. 1) The mixed micelle is taken up into the cell intact with release of the lipid and bile salt once within the cell. 2) The mixed micelle interacts directly with the BBM during a "collision" between the micelle and BBM. Lipid subsequently partitions from the micelle into the cytoplasm while the bile salts of the mixed micelle are released into the UWL to again form mixed micelles with lipids in the intestinal bulk phase. 3) Lipid from the mixed micelle partitions into the aqueous phase of the UWL in close proximity to the BBM. Lipid subsequently diffuses into the cell across the BBM. As lipid enters the cell, more lipid partitions from the mixed micelle into the bulk phase thereby becoming available for permeation through the BBM. In this model, the bile acid micelle acts as a solubilizer to convey relatively insoluble lipid molecules across the UWL.

Experimental support for the third model was provided by a study by Westergaard and Dietschy [5]. In this study, the uptake of different concentrations of fatty acids and cholesterol in the presence of varying concentrations of the bile salt taurodeoxycholate was studied in an in vitro rabbit jejunal preparation. If the first or second theory of lipid uptake from the micelle is correct, lipid uptake should increase as the amount of lipid present in the micellar phase increases. If the third model is correct, increasing the amount of fatty acid present in the micellar phase as compared to that present in the aqueous phase should decrease fatty acid uptake.

They demonstrated that increasing the amount of fatty acid and bile salt present in equal proportions did not increase the rate of lipid uptake by the tissue. If the second model was correct, more lipid uptake would have been predicted since more mixed micelles would be present to "collide" with the BBM. Increasing the bile acid concentration while keeping the fatty acid concentration constant resulted in decreased fatty acid uptake. This result is consistent with the third model since increasing the bile acid concentration without increasing the fatty acid concentration results in increased micellar lipid concentration and a decreased aqueous phase lipid concentration. Thus, the mixed micelle dissociates into lipid and bile salt while still within the aqueous compartment of the UWL with lipid uptake from the mixed micelles being proportional not to the mass of lipid in the micellar phase but to the mass of lipid present in the aqueous phase. The bile acid micelles serve as reservoirs for the hydrophobic lipids from which lipids partition into an obligatory aqueous phase prior to uptake by the BBM.

A recent study using brush border membrane vesicles (BBMV) prepared from rabbit jejunum has challenged this proposed mechanism of lipid uptake. Proulx et al [6] studied the influence of taurocholate on the uptake of [^{3}H] cholesterol from micelles of monoolein and oleic acid. They demonstrated that uptake of [^{3}H] cholesterol over time was greater in the presence of taurocholate than under similar conditions in the absence of the bile acid. This stimulatory effect increased in linear fashion as the concentration of taurocholate approached 20 mM with the cholesterol concentration remaining constant. As both cholesterol and taurocholate concentrations were increased with a constant concentration ratio, cholesterol uptake increased linearly. Stirring the solutions in which the cholesterol-containing bile salt free micelles were incubated did not alter uptake by the BBMV suggesting that the UWL resistance was not significant in this preparation.

Since lipid uptake increased in the presence of bile acid and increased as the bile acid concentration increased, bile acids may directly stimulate uptake of lipids by the BBM. Therefore, not only does the

BBM absorb lipid from an aqueous phase, but it must also absorbs lipid from a micellar phase. Therefore, bile acids do not function exclusively as reservoirs of hydrophobic lipids prior to their transfer to the aqueous phase for absorption across the BBM.

Nonetheless, it must be concluded, the uptake of lipid from the aqueous intermediate phase in close proximity to the UWL constitutes an critical step in lipid absorption by the BBM. What is the mechanism that "triggers" this dissociation?

In a 1975 study, Lucas et al [7] used pH microelectrodes to determine the pH of the bulk phase solution and the mucosal surface "microclimate" in a preparation of everted rat proximal jejunum. A region of low pH was detected adjacent to the jejunal mucosa with an estimated pH of at least 5.5 while the intraluminal buffer pH was 7.2. They postulated that the failure to produce this microclimate of low pH may explain certain malabsorption syndromes.

In a subsequent study, Shiau and Levine [8] examined the diffusion of radiolabelled taurocholate and oleic acid from a micellar solution into a phosphate buffer with a pH varying from 5.0 to 8.0. As the pH of the micellar solution decreased, the rate of diffusion of oleic acid from the micellar solution into the phosphate buffer solution increased. They postulated that the low pH compartment adjacent to the BBM detected by Lucas et al [7] plays an important role in lipid-bile acid dissociation from the mixed micelle. Once released from the mixed micelle into the acidic microclimate, fatty acids become protonated and preferentially partition into the lipid membrane of the BBM.

Shiau [9] further examined the effect of pH in the microclimate adjacent to the BBM on micellar oleic acid uptake in segments of everted rat proximal jejunum. Increasing the microclimate pH to 6.47 resulted in decreased oleic acid uptake as compared to uptake under basal conditions with a microclimate pH of 6.05. Thus, lipid uptake from mixed micelles was enhanced by an acidic microclimate adjacent to the BBM.

Subsequently, Shiau et al [10] isolated an acidic mucin located adjacent to the intestinal epithelium in the pig. Upon exposure to this mucin, mixed micelles of oleic acid and taurocholate dissociated. This supported the theory that a low pH compartment adjacent to the intestinal epithelium is critical for the dissociation of mixed micelles and the subsequent absorption of lipid by the small intestinal epithelium.

In summary, the UWL forms a barrier to uptake of lipid from the intestinal lumen. Movement across the UWL is passive and resistance to movement of a particular probe molecule across it depends upon the functional thickness of the UWL, the aqueous diffusion constant of the molecule and the concentration gradient between the bulk water phase and the underlying BBM. Bile acid micelles facilitate movement of hydrophobic lipids across the UWL by solubilizing them, thereby forming mixed micelles. In the acidic microclimate adjacent to the BBM, mixed micelles dissociate with subsequent protonation and transit into the BBM of the lipid molecules and the release of the bile salts from the mixed micelle into the intraluminal bulk phase. Potentially, there may be direct interaction between mixed micelles and the BBM resulting in BBM uptake of lipid.

After traversing the UWL, the lipid molecule has one final barrier, the BBM, to traverse before it can enter the enterocyte. In the next section, lipid transit across the BBM will be reviewed.

5.4 LIPID MOVEMENT ACROSS THE BRUSH BORDER

Early studies of lipid uptake by the small intestine were hindered by the inability to separate the effects of UWL resistance from BBM resistance. A landmark study of lipid uptake by the small intestine which quantified the resistance of these two barriers separately was a 1973 study by Sallee and Dietschy [11].

In this in vitro study, radiolabelled short and medium chain saturated fatty acids and alcohols in concentrations of 1 mM were incubated with everted rat jejunal sac preparations. Following incubation, the intestinal segments were dried and tissue uptake of radiolabelled solute was determined.

UWL thickness was determined by measuring the half time ($t\frac{1}{2}$) for the evolution of an electrokinetic potential across the intestinal segments upon exposure to probe molecules. To determine the effect of the UWL on fatty acid and alcohol uptake, uptake was determined during vigorous stirring of the mucosal solution, to reduce the effective thickness of the UWL, and while the mucosal solution was undisturbed. Uptake was assessed at varying temperatures, in the presence and absence of bile salt (20 mM taurodeoxycholic acid), at pH 7.4 and 6.0 and at varying times of incubation. As well, uptake of radiolabelled solute was determined in the presence of other fatty acids or alcohols and in the presence of the metabolic inhibitors iodoacetamide and carbonyl *m*-chlorophenyl hydrazone. These metabolic inhibitors cause as much as 99% inhibition of intracellular metabolic processes such as CO_2 production and fatty acid and sterol synthesis.

Preliminary studies of UWL thickness using the probe molecules urea, pentaerythritol, mannitol, sucrose, raffinose, glycine and β alanine demonstrated a thickness of 182 to 207 μm. The mucosal uptake of radiolabelled saturated fatty acids 2:0 through 12:0 and the saturated alcohols 6:0 through 10:0 increased linearly with respect to time up to 4 minutes incubation. As well, tissue uptake was linear with respect to bulk phase concentration, with no evidence of saturation kinetics to suggest carrier-mediated transport. Uptake was not influenced by the presence of related substances or metabolic inhibitors and was not influenced significantly by variation in temperature. Tissue uptake of fatty acids and alcohols increased as the length of the fatty acid chain increased and was higher at pH 6.0 than at pH 7.4. Stirring the mucosal solution to decrease UWL thickness had little effect on mucosal uptake of the 7:0 and 8:0 fatty acids, but significantly increased uptake of fatty acids 10:0 and 12:0. Bile acids did not affect the uptake of 7:0 and 8:0 fatty acids. However, as fatty acid chain length increased beyond 8:0, there was a progressive lowering of tissue uptake.

Thus, fatty acids and alcohols are absorbed across the BBM via simple passive diffusion. Permeability coefficients for the absorption of these compounds were calculated as nmoles of radiolabelled compound uptake per 100 mg dry tissue weight of mucosa per minute with data normalized to a 1 mM concentration of the test fatty acid or alcohol. The permeability coefficients increased with increasing chain length with higher permeability coefficients recorded at pH 6.0 as compared to pH 7.4. Movement across the BBM was the rate limiting step for the uptake of short and medium chain fatty acids. Movement across the UWL would be predicted to be the rate limiting step in the absorption of long chain fatty acids. After correction of the permeability coefficients of the 7:0 to 12:0 fatty acids for the UWL resistance, the true permeability coefficients increased by an average of 1.78 for each $-CH_2-$group added to the fatty acid chain. This corresponded to an overall incremental free energy change, defined as the change in energy that occurs when a methylene group partitions from the luminal environment into the brush border membrane, $\delta\Delta F_{w\rightarrow l}$ of -1489 joules·mole^{-1} (-356 calories·mole^{-1}).

Subsequent studies supported the concept that movement of lipid into the cell was passive.

Westergaard and Dietschy [12] studied the effects of the UWL and the BBM on the uptake of saturated fatty acids, saturated alcohols and bile acids from solutions containing 0.25-1.0 mM concentrations of the test molecules in an in vitro rabbit preparation. Segments of jejunum were mounted in incubation chambers and the effective thickness of the UWL controlled by varying the rate at which the solution bathing the mucosal surface of the jejunum was stirred. UWL thickness was calculated by measuring the change in the transmural potential difference that occurred during exposure of the jejunal mucosa to a hyperosmolar solution.

UWL thickness was decreased with increased mucosal solution stirring. Following 5 minutes of incubation, the calculated UWL thickness was 334 µm in the unstirred state and decreased to an essentially constant value of 160 µm at stirring rates exceeding 400 rpm. Uptake of the saturated fatty acids 4:0 through 12:0 and the saturated fatty alcohols 6:0 through 12:0 by jejunal segments was determined under unstirred conditions as well as while the mucosal solution was stirred at 600 rpm.

Studies of uptake under stirred and unstirred conditions demonstrated that the limiting factor for the uptake of long chain fatty acids and alcohols was the unstirred water layer resistance. The permeability coefficient (see above) increased by a factor of 1.52 with an incremental free energy change of -258 $\mathrm{cal \cdot mol^{-1}}$ for each CH_2 group added to the fatty acid chain.

Using an in vivo rat preparation, Westergaard [13] showed that uptake of saturated fatty acids of chain length 4:0, 6:0, 8:0, 9:0, 10:0 and 12:0 increases linearly over the concentration range 0 to 8.0 mM for each fatty acid. This confirmed that fatty acid uptake across the UWL and BBM is via passive diffusion.

Using BBMV to examine uptake of lipids by the BBM has allowed researchers to study movement across this membrane in isolation without the effects of cytosolic lipid-binding proteins or the UWL. Further details regarding the preparation and advantages of BBMV in the study of nutrient uptake by the BBM can be found in the chapter on glucose uptake.

Pioneering studies of lipid uptake by small intestinal BBMV were carried out by Proulx et al. In a 1982 paper, Proulx et al [14] examined the uptake by rabbit jejunal BBMV of several lipids, fatty acids and cholesterol. Uptake during 15 minute incubations of radiolabelled lipids was determined at different pH values from pH 3.5 to 10.0. The effects of Ca^{2+} in varying concentrations, several other divalent ions, K^+ and Na^+ on uptake were studied by measuring uptake both in the presence and absence of these ions.

Lipid uptake by the BBMV did not conform to saturation kinetics and increased linearly with increased lipid in the incubation medium over the concentration range 0 to 1.5 μmol/mL incubation buffer. Uptake was enhanced when Ca^{2+} ions and other divalent cations were added to the incubation medium but not when monovalent cations were added. Lipid uptake by vesicles was optimal in the pH range 6.5 to 8.0.

In a second study, Proulx et al [15] incubated BBMV isolated from either the whole small intestine or from different segments of the small intestine with radiolabelled oleic (18:1) or palmitic acid (16:0) in concentrations from 0.1 mM to 0.4 mM in the presence of taurocholic acid and Ca^{2+} ion. In a second part of the study, uptake of radiolabelled fatty acid by vesicles prepared from whole small intestine was determined for caproic (6:0), lauric (12:0), palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acid each in a concentration of 0.8 mM. Fatty acid uptake was also determined in hyperosmolar solution induced by the addition of 300 mM cellobiose to the incubation medium. In these circumstances, the intravesicular space would be eliminated due to shrinkage of the vesicles and lipid uptake would represent uptake by the vesicular walls exclusively.

Similar results were noted for the uptake of palmitic and oleic acid in vesicles prepared from the jejunum, ileum or whole small intestine. Uptake was lower for both fatty acids in vesicles isolated from the duodenum. Uptake of fatty acids into the BBMV was greater for longer chain fatty acids than shorter chain fatty acids. Fatty acid uptake was not affected by treatment of vesicles with 300 mM cellobiose suggesting that lipid uptake does not involve the intravesicular aqueous space. Binding of lipid increased with chain length up to 16 carbons, suggesting that uptake relates directly to the relative solubility of the fatty acids in the apolar phase of the membrane. As in previous studies, Ca^{2+} enhanced fatty acid uptake of the BBMV. Taurocholic acid in concentrations of 1 to 5 mM in the incubation medium stimulated fatty acid uptake. At higher concentrations of this bile acid, the membrane vesicles were dissolved resulting in decreased fatty acid uptake.

In summary, lipid movement across the BBM has been studied using everted jejunal sac preparations, in vivo small animal preparations and in vitro preparations of isolated pieces of small intestine. As well, BBMV have been used to study BBM lipid uptake. These studies have demonstrated that the UWL resistance influences lipid uptake with resistance being particularly important in the uptake of fatty acids with fatty acid chain length over 8:0. Studies using BBMV have demonstrated that lipid uptake is inhibited in the absence of Ca^{2+} ion but not Na^{+} ion. Uptake is not inhibited in the presence of metabolic inhibitors and is not temperature sensitive. Uptake in the *in vitro* state does increase with a decrease in mucosal pH.

These studies suggest that fatty acid uptake across the intestinal mucosa is passive. Saturable kinetics were not identified using either a whole tissue preparation or BBMV. However, several more recent studies have suggested that lipid uptake may not be entirely passive. In the next section, studies suggesting that movement of lipids across the BBM may be protein mediated and not completely passive will be reviewed.

5.5 LIPID UPTAKE ACROSS THE BRUSH BORDER: PROTEIN MEDIATED?

The studies reviewed above have suggested that lipid uptake by the BBM is a passive process. Lipid uptake is independent of temperature, the presence of metabolic inhibitors or the presence of similar compounds that might serve as "competitors" for an active carrier. However, although lipid uptake does not depend on the presence of Na^{+} ions, it is stimulated in the presence of Ca^{2+} ions. Thus, is there a carrier mediated component to lipid uptake by the BBM?

A 1972 study by Ockner et al [16] examined the uptake of the unsaturated fatty acid linoleic acid (18:2) and the saturated fatty acid palmitic acid (16:0) in a rat *in vivo* and *in vitro* everted jejunal sac preparation. *In vivo*, following intraduodenal infusion of radiolabelled fatty acid, linoleic acid (LA) was absorbed more completely in the proximal small intestine than palmitic acid (PA). However, in the everted jejunal sac preparation, uptake of PA equalled or exceeded that of LA. Uptake of both fatty acids by the everted sac preparation conformed to saturation kinetics as fatty acid uptake did not increase proportionately with increasing concentration in the mucosal solution. Uptake of varying concentrations of fatty acid by everted jejunal sacs after 1 minute incubation was determined and, using linear regression, the mean reciprocal of uptake in micromoles per gram of sac was plotted against the mean reciprocal of fatty acid concentration in millimolar. Using this method, virtually identical values for V_{max} of 1.45 μmoles per gram of sac per minute for palmitic acid and 1.47 μmoles per gram of sac per minute for linoleic acid were calculated with a K_m of 5.3 mM for PA and 6.6 mM for LA.

They postulated that the uptake from the intestinal lumen of LA was faster than that of PA due to one or more of the following factors: (1) PA is less readily incorporated into micelles and therefore is less soluble in jejunal contents; (2) uptake of PA by jejunal absorptive cells is slower than uptake of LA; (3) PA, once taken up, is less rapidly reesterified into complex lipids by the absorptive cell. Possibly a membrane lipid carrier might be present, although they considered this less likely in view of their inability to demonstrate competition for the uptake process. Saturation kinetics might be explainable by limited solubility of the fatty acid in the plasma membrane. Nonetheless, they suggested that fatty acid uptake occurs by a process more complex than simple passive diffusion.

Subsequent studies of lipid uptake by the small intestine have focused on the study of LA uptake. LA is the major dietary essential fatty acid in man. Synthesized readily by plants, it cannot be readily synthesized by mammals. Absorption of LA is critical since deficiency of this fatty acid results in reduced growth rates, skin disorders, increased platelet aggregation, decreased prostaglandin synthesis and reduced myocardial contractility. This essential fatty acid also serves as a structural component of cell membranes.

In two 1979 studies, Chow and Hollander [17,18] examined LA absorption in the rat. In an in vitro everted jejunal sac preparation [17], they studied the uptake of varying concentrations of radiolabelled LA in a standard phosphate buffer solution at pH 6.5 with 10mM sodium taurocholate.

Plotted against time of incubation up to 6 minutes, uptake of LA increased linearly with time. Uptake was not influenced by the presence of metabolic inhibitors and uncouplers and was similar at incubation temperatures of 37, 28 and 20·C. The absorption rate was significantly increased by increasing the rate of stirring of the mucosal solution and increased as mucosal solution pH decreased.

At LA concentrations between 42μM and 1260 μM, analysis of the rate of absorption at each concentration plotted against the concentration suggested that LA was absorbed by a saturable mechanism. Using a Hofstee plot, V_{max} and K_m of 125 nmol/min per 100 gm of tissue and 92 μm respectively were calculated. By contrast, at luminal concentrations of 2.52 to 4.2mM, the rate of LA absorption versus its concentration fitted best to a linear plot suggesting that uptake of LA is passive at higher luminal concentrations.

Since LA uptake was not influenced by temperature, the presence of the oxidative phosphorylation uncoupler 2,4-dinitrophenol or the cytochrome c-oxidase inhibitors potassium cyanide and sodium azide, uptake of LA does not require metabolic energy. However, the presence of saturation kinetics suggested that, at low luminal concentrations, LA is absorbed by a carrier mediated, facilitated diffusion mechanism.

In a subsequent in vivo study [18], segments of jejunum in conscious restrained rats were perfused with solutions containing radiolabelled LA and 10 mM sodium taurocholate in phosphate buffer solution. Aliquots of the perfusate were taken both before and after perfusion through the segments and absorption from the segment calculated from the decrease in specific activity of the radiolabelled LA. Absorption rates of LA were determined with varying concentrations of LA, in the presence of the unsaturated long chain fatty acids oleic (18:1), linolenic (18:3) and arachadonic (20:4) and the phospholipid lecithin, when Na^+ ions in the phosphate buffer were replaced by K^+ ions, in the presence of varying concentrations of the bile acid sodium taurocholate and in the presence of the nonionic surfactant, Tween 80. To determine the effect of the UWL on LA uptake, the perfusion rate was varied, with higher rates of perfusion resulting in decreased UWL thickness.

Similar to the results of their in vitro study [17], these studies demonstrated that absorption of LA delineates apparent saturation kinetics at intraluminal concentrations between 21 and 1260 μM. At higher concentrations, the relationship between concentration and absorption rate is linear suggesting that passive absorption is occurring.

LA absorption was significantly decreased in the presence of linolenic, oleic or arachidonic acid in the perfusate. Uptake also decreased in the presence of lecithin. As the pH of the perfusate decreased, LA uptake increased. As bile salt concentration increased, uptake of LA also increased. Addition of Tween 80 decreased LA uptake as did the substitution of K^+ ion in the perfusate for Na^+ ion. Uptake of LA increased in parallel with a decrease in the perfusion rate.

These results suggested that facilitative absorption is the predominant mechanism of LA absorption at low intraluminal concentrations while passive diffusion predominates at higher intraluminal concentrations. The decreased LA absorption in the presence of other fatty acids may be due to enlargement of the bile acid-fatty acid mixed micelles with resultant decreased diffusion rate towards the BBM or due to competition by the other fatty acids for binding to a FABP on the cytosolic side of the BBM. Lecithin inhibition of LA uptake similarly may have been secondary to micellar enlargement. Decreased lipid absorption in the absence of Na^+ ion may have been secondary to Na^+ ions being necessary for the formation of a complex between LA and its carrier.

Ling et al [19] examined the uptake of LAd by rabbit jejunal and ileal BBMV. Uptake of radiolabelled LA was determined in the presence and absence of Na^+ ions, under conditions of varying extravesicular osmolarity, and in the presence of varying concentrations of the bile acid taurocholic acid. LA uptake by the vesicles was determined as a function of varying time of incubation and varying concentrations of LA in the incubation solution. Uptake was determined in the presence and absence of phloretin and under conditions of varying temperature of incubation.

LA uptake increased linearly with increasing concentrations from 10 to 100 μM. When measured over time, rapid uptake of LA occurred during the first 15 seconds of incubation. Uptake of LA was unaffected by variation of incubation medium osmolarity between 240 mOsm and 820 mOsm suggesting that LA accumulation was mainly due to binding of the fatty acid to the BBM rather than due to intravesicular accumulation. Intravesicular accumulation of LA was determined by incubating vesicles with LA and then lysing them with ice cold distilled water. These trials suggested that approximately 40% of the LA taken up by the vesicles had accumulated in the intravesicular space. LA uptake was not affected by lowering the temperature of incubation from 21° to 4°C nor was it influenced by the presence or absence of Na^+ ions in the incubation solution. Uptake was unaffected by known inhibitors of anion transport; diisothiocyanatostilbene and isothiocyanatostilbene.

However, LA uptake was partially inhibited by the addition of phloretin, which is able to inhibit specific membrane proteins. As well, an "overshoot" of LA uptake was noted during the first minute of the time course study which was not explained. The authors suggested that LA uptake, in the concentrations studied, was passive. However, they also suggested that the absorption of LA in vivo could be partly due to a carrier mediated facilitated transport mechanism. Inhibition of uptake by phloretin suggested the involvement of a membrane protein in the initial uptake of LA by the BBM.

Conflicting results were noted in a similar study of LA uptake by rabbit jejunal BBMV by Keelan et al [20]. When ice-cold deionized water was added to vesicles previously incubated with LA, there was no significant release of LA, suggesting that there was no intravesicular accumulation of the fatty acid. When LA uptake was studied as a function of time, an "overshoot" was not demonstrated in the presence of a Na^+ ion gradient. LA uptake increased linearly with increasing LA concentrations when carried out under initial rate conditions. These findings were compatible with a process of passive uptake of LA with partitioning of the fatty acid into the BBM. Vesicular uptake of LA was unaffected by the absence of Na^+ ion, the presence of a Na^+ ion gradient across the vesicular wall or varying osmolarities of the buffer. However, it was inhibited to 88% of control values by the addition of 0.6 mM phlorizin and to 58% of control values by the addition of 0.2 mM phloretin to the incubation medium. Thus, although this data did not demonstrate carrier mediated facilitated transport of LA, possible physiological importance of a brush border membrane FABP was not eliminated.

Several subsequent studies have also suggested the possible existence of protein mediated transport of lipids across the brush border membrane. Thurnhofer and Hauser [21] studied cholesterol uptake by rabbit BBMV both with and without proteolytic treatment of the vesicles with papain. Papain treatment resulted in release of about 25% of the total membrane protein and a dramatic change in both the amount and character of cholesterol uptake by membranes.

Saturation kinetics were demonstrated for cholesterol uptake by untreated BBMV. However, following proteolytic therapy, cholesterol uptake was significantly reduced and uptake changed from a second order reaction to a first order reaction which is more representative of a passive diffusion mechanism. Protein released from the BBM following papain digestion was able to bind cholesterol. The authors therefore concluded that the BBM absorbs cholesterol via a protein mediated facilitated process.

Similar techniques were used to study the uptake of phosphatidylcholine by the small intestinal BBM [22]. As with cholesterol, phosphatidylcholine uptake by the BBM was protein mediated and saturable. As well, uptake could be inhibited by enzyme inhibitors such as *N*-ethylmaleimide, $CuSO_4$, 5-dimethylamino-1-napthalenesulfonyl chloride and 3-isothiocyano-1,5-naphthalenedisulfonic acid. Protein released from the BBM following papain digestion was able to bind phosphatidylcholine.

In a subsequent paper from the same laboratory, using gel filtration and affinity chromatography techniques, Thurnhofer et al [23] were able to purify and characterize a cholesterol and phosphatidylcholine transferring protein in rabbit small intestine BBM. This protein was isolated and has an apparent molecular weight of between 12000 and 14000.

These studies therefore support the concept that lipid uptake by the BBM is protein mediated. Further support for this concept has been provided by the studies from the laboratory of Stremmel et al [24, 25].

In 1985, Stremmel et al [24] studied oleic acid (OA) uptake by rabbit BBMV. Uptake of OA was pH sensitive with maximal uptake occurring at pH 4.0. Uptake could be reduced at pH 8.0 by the pretreatment of vesicles with trypsin. However, trypsin did not influence uptake at pH 4.0. This finding suggests that, at low pH, a nonionized OA molecule binds preferentially to membrane lipids while at physiologic pH, the fatty acid binds to a protein binding site. Radiolabelled OA uptake in the concentration range 100 to 4500 nM was determined for both heat denatured and native BBMV. Uptake for heat denatured vesicles increased linearly as a function of increasing OA concentration suggesting nonspecific binding. When this uptake was subtracted from the uptake by native membranes, an uptake curve representative of heat sensitive, saturable and specific binding was obtained.

Thus, there was protein mediated OA uptake by the BBM. In a second part of the study, affinity chromatography over OA-agarose columns of solubilized proteins from rat jejunal BBM was performed which identified a 40000 mol wt protein as the OA BBM binding protein. This protein not only co-chromatographed with OA but also chromatographed with the long chain fatty acids palmitate, arachidonate and linoleate. It did not co-chromatograph with the oleate ester of cholesterol, phosphatidylcholine, taurocholate or sulfobromophthalein. Double immunodiffusion studies demonstrated a single precipitin line of identity between this protein and an antibody raised against rat liver plasma membrane fatty acid binding protein (LPM-FABP).

In a final section of this study, rat jejunal mucosa was incubated with rabbit anti LPM-FABP. Immunofluorescence studies of the jejunal mucosa demonstrated the presence of LPM-FABP in the apical parts of enterocytes of the crypts and villi. It was also seen in the lateral cell borders in the region of the tight junctions. In the presence of anti LPM-FABP, binding of fatty acids to the BBM was selectively inhibited.

Stremmel later studied OA uptake in isolated rat jejunum mucosal cells and demonstrated that uptake was saturable and temperature-dependent [25]. He calculated a K_m of 93 $\pm$ 8.9 nM and a V_{max} of 2.10 $\pm$ 0.19 $nmol \cdot min^{-1}$ per 10^6 cells. Uptake of OA was inhibited in the absence of Na^+ ions in the incubation medium. Pre-treatment of the cells with an antibody to the membrane FABP inhibited uptake of OA as well as the uptake of linoleate, arachidonate, palmitate and D-monopalmitin. Uptake of L-alanine was not affected by this antibody pretreatment.

In a second section of this study, OA uptake in an isolated segment of rat jejunum was studied. The segment was perfused intraluminally with $[^3H]$ OA and Na^+ taurocholate. Uptake of $[^3H]$ OA was determined in the presence of various short, medium and long chain fatty acids, with varying concentrations of OA, in the presence and absence of intraluminal Na^+ ion and after the luminal segments had been perfused intraluminally with antibodies to the membrane FABP.

Uptake at high intraluminal concentrations (1-3 mM) increased linearly as the concentration of OA increased, suggesting a passive diffusional transport mechanism. However, at low OA concentrations (0.05-0.5 mM), uptake was saturable with high carrier affinity as evident by a K_m of 0.271 mM with a V_{max} of 8.125 $nmol \cdot cm^{-1} \cdot min^{-1}$. Uptake was reduced in the presence of long chain fatty acids, fatty acid methyl ester, monoglyceride , L-lysophosphatidylcholine and cholesterol but was unaffected by the presence of short and medium chain fatty acids, D-glucose or alanine in the mucosal solution. Oleic acid uptake in both the in vitro and in vivo preparations decreased in the absence of Na^+ in the mucosal incubating solution. As well, uptake was inhibited by the addition of ouabain, which inhibits Na^+/K^+ ATPase activity in the basolateral membrane, to the mucosal solution. Decreased uptake was noted in jejunal segments pretreated with FABP antibody.

These studies supported the concept that there is a protein mediated component to OA uptake. There was competitive inhibition of OA uptake in the presence of other long chain fatty acids. Stremmel suggested that either these molecules compete with OA for binding sites on the FABP or they enlarge the micelles in the mucosal solution with resultant reduction in the diffusion rate of OA across the UWL. Short and medium chain fatty acids do not affect OA uptake suggesting that either these molecules do not interfere with micellar formation or they are taken up by different transport mechanisms. Stremmel postulated that there may be primary Na^+ dependent lipid transport across the BBM. However, an alternate explanation of the role of the Na^+ ion is that the Na^+ ion may be necessary to maintain transmembrane ionic gradients and induced electrical potential differences as driving forces of lipid transport. Inhibition of OA uptake by pretreatment of the intestine with FABP antibody provided further evidence of protein mediated lipid uptake by the BBM.

In further studies, using a similar in vitro and in vivo model as used in the previously described studies, Stremmel and Hoffmann [26] examined the uptake of the unconjugated dihydroxy bile acids deoxycholic and chenodeoxycholic. They demonstrated that uptake of these two bile acids was of first order kinetics and not influenced by the simultaneous uptake of OA or the presence of an antibody to the previously described FABP. Thus, absorption of unconjugated dihydroxy bile acids in the rat jejunum does not involve the same carrier mediated uptake mechanism as is involved in the uptake of long chain fatty acids. Uptake of these compounds likely is by passive nonionic diffusion.

Thus, these two research groups demonstrated carrier mediated lipid uptake by the BBM of the small intestine. In studies from Hauser's laboratory [21,22,23], uptake of cholesterol and phosphatidylcholine was studied in the rabbit with identification of a carrier protein with a molecular weight of between 12000 and 14000. Studies from Stremmel's laboratory [24,25] on long chain fatty acid uptake in the rat established that uptake involved a 40000 molecular weight protein able to bind long chain fatty acids but not oleate ester of cholesterol, taurocholate, phosphatidylcholine, sulfobromophthalein or unconjugated dihydroxy bile acids.

In summary, lipid uptake by the BBM occurs by two mechanisms: a passive diffusional process at high intraluminal lipid concentrations and a carrier protein mediated component at low intraluminal concentrations. These carrier proteins have been identified; a 40000 mol wt protein for the transport of long chain fatty acids and a protein of between 12000 and 14000 mol wt protein for the transport of cholesterol and phosphatidylcholine. Carrier mediated lipid uptake is saturable, specific, influenced by changes in pH and temperature, can be inhibited by metabolic inhibitors, demonstrates competitive inhibition in the presence of similar molecules and decreases in the absence of Na^+ ions.

Researchers have examined the effect of lipids on the physical and absorptive characteristics of the BBM. In the next section, the influence of dietary fats on BBM function will be reviewed.

5.6 INFLUENCE OF DIETARY FATS ON UPTAKE PROPERTIES OF SMALL INTESTINE

Absorption of fatty acids and cholesterol is associated with their incorporation into the lipid bilayer of the brush border membrane. Does the absorption of dietary lipids change the properties of the brush border membrane and, if so, can dietary manipulation be used to control the absorptive capacities of the small intestine? Researchers have investigated these questions with resultant data suggesting that dietary fat manipulation can alter enterocyte nutrient absorption properties.

Thomson [27] examined the influence of diet on the in vitro uptake by rabbit intestine of D-glucose, cholesterol and a homologous series of saturated fatty acids and fatty alcohols. In this study, rabbits were weaned and fed standard rabbit chow or a diet containing 2% fat, 0.2% cholesterol or 0.2% cholesterol plus 2% fat for 6 weeks. They were then sacrificed and uptake by segments of jejunum and ileum of radiolabelled substrate determined.

Feeding of the test diets was associated with marked increases in the uptake of saturated fatty acids and alcohols but a decline in the uptake of cholesterol. Qualitatively, the relative rates of uptake of the fatty acids and alcohols in the diet groups were 0.2% cholesterol > 0.2% cholesterol plus 2% fatty acids = 2% fat > control chow diet. Uptake of glucose was greater in rats fed a diet containing either 2% fat or 0.2% cholesterol with a linear relationship between glucose concentration and uptake as compared to the curvilinear relation between glucose concentration and uptake noted in the control rats and rats fed a diet containing 2% fat plus 0.2% cholesterol.

This data supported a model of the BBM as being heterogenous with regards to the diffusion pathways for different lipids; since diet influenced fatty acid uptake in a different way than it influenced cholesterol uptake. Thomson postulated that modifications of dietary lipid content could modify glucose uptake as a result of changes induced in the maximal transport rate, the contribution of passive permeation to glucose uptake or resistance of the UWL to glucose uptake. He proposed that dietary lipid modifications are associated with reciprocal changes in the effective resistance of the UWL and the passive permeability characteristics of the intestine as well as alterations in the kinetic constants of the intestinal uptake of glucose.

In a subsequent study, Thomson et al [28] determined the in vitro jejunal uptake of cholesterol, fatty acids, decanol, bile acids, glucose, galactose and leucine in rats fed either standard rat chow or a semipurified diet containing 20% fat of either a high or low polyunsaturated-to-saturated fatty acid ratio for 2-3 weeks prior to sacrifice. Substrate uptake was determined in isolated segments of jejunum. BBMV were prepared from jejunal segments and analyzed to determine the effects of the different diets on the BBM free fatty acid, bile acid, cholesterol, cholesterol ester and phospholipid content.

Uptake of myristic acid (14:0) was unaffected by dietary manipulation. However, uptake of PA (16:0), stearic acid (18:0) and cholesterol were greater in rats fed a high saturated fat diet than in rats fed a high polyunsaturated or chow diet. Compared to rats fed a high polyunsaturated fat diet, rats fed a high saturated fat diet had greater jejunal uptake of decanoic acid (10:0), dodecanoic acid (12:0), and LA (18:2). Uptake of cholic acid and glychocolic acid was not influenced by diet. However, uptake of chenodeoxycholic acid and taurochenodeoxycholic acid was lower in rats fed a high saturated fat diet as compared to rats fed a high polyunsaturated fat diet. Carrier mediated transport of D-glucose in the jejunum was influenced by diet with uptake being greater in rats on a high polyunsaturated diet.

Analysis of BBM enzyme and lipid composition demonstrated that rats fed a high polyunsaturated or saturated cholesterol diet had higher alkaline phosphatase (ALP) but lower invertase (INV) activity as compared to rats fed a chow diet. The BBM ratio of total phospholipid/total cholesterol was lower in the ileum as compared to the jejunum only in animals fed a high saturated fat diet, Otherwise, there were no differences in the three groups with regards to BBM content of total free fatty acids, total bile acids, total phospholipids, total free or cholesterol esters or the ratio of total phospholipids/total cholesterol. Morphology studies of the small intestine demonstrated that feeding rats a high saturated fat diet abolished the morphological differences between the ileum and jejunum seen in the other two groups of rats.

Thus, changes in the fatty acid composition of the diet could influence the uptake of passively and actively transported solutes. Proposed mechanisms for these changes included via changes in the morphology of the gut, changes in the chemical composition of the BBM, changes in the function of carrier proteins or changes in the characteristics of the outer diffusion barriers.

Thus, dietary fat can influence nutrient uptake by the BBM. However, the mechanisms by which this occurs are unclear. Subsequent studies have attempted to determine the mechanisms by which diet can influence nutrient uptake by the BBM.

In an in vitro study of hexose and lipid uptake in rats, Thomson et al [29] demonstrated that dietary fat manipulation affected uptake of lipids and the hexoses glucose, galactose and 3-0 methyl glucose. They noted that UWL resistance was affected by diet with greater resistance noted in rats fed a high fat diet as compared to rats fed a low fat diet.

Although they believed that altered UWL resistance in response to dietary manipulation could influence nutrient uptake, they did not believe that the changes in nutrient uptake that they noted could be explained entirely by changes in UWL resistance. Dietary change could result in a change in the functional surface of the BBM able to absorb nutrients. Possibly, dietary modifications result in a change in the membrane phospholipid fatty acyl chain composition with secondary changes in the permeability properties of the membrane itself.

Merrill et al [30] studied the uptake of Ca^{2+} ion by BBMV incubated with different fatty acids and methyloleic acid. OA and LA stimulated at lower and inhibited at higher concentrations the uptake of Ca^{2+} while methyloleic acid stimulated Ca^{2+} uptake over the entire range of concentrations tried. PA did not affect Ca^{2+} uptake.

The authors suggested that uptake of lipids modified the composition of the BBM with resultant effect on its structural relationships or domains. Fatty acids, they postulated, were taken up by lipid domains within the BBM. Ca^{2+} uptake was influenced by these fatty acids when the Ca^{2+} transport channels interacted with these domains.

Brasitus et al [31] studied proximal small intestine BBMV uptake of D-glucose in rats maintained for 4 weeks on nutritionally complete diets enriched in unsaturated (fish oil) or saturated (butter fat) triglycerides. As well, they determined BBM fluidity and fatty acid composition in each group.

Neither diet altered the neutral lipid or phospholipid composition of the BBM. However, membranes prepared form animals fed the unsaturated fat enriched diet contained greater amounts of saturated fatty acids as well as (n-3) unsaturated fatty acids and lower percentages of monounsaturated and (n-6) unsaturated fatty acids than membranes prepared form rats fed a saturated fat enriched diet. Membrane fluidity was greater in membranes prepared form animals maintained on a saturated fat enriched diet. Na^+-gradient dependent D-glucose transport was affected by diet with a significantly greater V_{max} calculated for BBMV prepared from rats fed an unsaturated fat enriched diet as compared to rats fed a saturated fat enriched diet. There was no significant difference between the groups with regards to the K_m for D-glucose transport.

Thus, dietary modification influenced the composition, physical properties and transport characteristics of the BBM. Membrane fluidity was influenced by diet. However, the authors did not believe that the differences in membrane fluidity were responsible for the changes in Na^+ gradient dependent D-glucose transport seen as a result of diet manipulation.

Tirruppathi et al [32] studied the transport of L-alanine, D-glucose and L-glucose and Na^+ ion flux across the BBM in vesicles prepared from rabbit small intestine which had been preincubated with oleic acid or ricinoleic acid. Active transport of these substrates was inhibited in vesicles preincubated with either fatty acid in the presence of an inwardly directed Na^+ gradient across the vesicular wall. They also established that H^+ gradient-dependent dimethylamiloride-sensitive Na^+ uptake was inhibited in vesicles preincubated with fatty acids; ricinoleic acid being more inhibitory than oleic acid. They suggested that fatty acids inhibit the Na^+-H^+ exchanger in the brush border membrane. By doing so, they influence the active transport of nutrients indirectly by collapsing the Na^+ gradient across the vesicular wall. Fatty acid inhibition is greater for unsaturated fatty acids such as ricinoleic acid (18:1-OH) than saturated fatty acids such as oleic acid (18:1). They postulated that this inhibitory effect of fatty acids may result from changes in the physical properties of the membrane with secondary altered function of membrane transport proteins.

Meddings and Theisen [33] examined the permeability of rat jejunal BBM to a series of fatty acids in the suckling, weaning and weaned animals. BBMV were prepared from rats of different ages and the chemical composition and fluidity of the BBM determined. The relationship between lipid permeability and the chemical composition and fluidity of the membrane at different ages was hence determined.

Lipid permeability decreased with age as the rats' diet changed from milk to milk plus chow and finally carbohydrate rich rat chow. Lipid permeability correlated directly with lipid fluidity in the BBM. The BBM was most hydrophobic in suckling rats with hydrophobicity declining with maturation.

Thus, BBM lipid permeability and fluidity decreased as the lipid content of the diet decreased. Of note, changes in the dynamic component of membrane fluidity were most marked in the superficial portions of the BBM lipid bilayer. In a subsequent review of membrane function [34], Meddings postulated that membrane fluidity regulates the lipid transport properties of the BBM and, by doing so, allows the animal to adapt to changes in dietary conditions.

In summary, nutrient uptake across the BBM changes in response to dietary lipid manipulation. The physical properties of the BBM change in response to diet with fluidity and lipid composition being sensitive to changes in the amount and nature of fat in the diet. As well, the UWL resistance is influenced by diet.

The mechanism by which diet influences nutrient uptake is as yet unclear. Changes in UWL resistance influence subsequent movement of compounds both passively and actively transported across the BBM. As well, dietary lipid may modify BBM structure and alter transporter function as a results of alterations in membrane fluidity or changes in the lipid microdomains in close proximity to membrane transporters. The exact mechanisms are unclear. Hopefully with further research, the mechanisms by which dietary manipulation influences nutrient uptake will be made clearer.

Once lipids have traversed the BBM, absorbed monoglycerates, cholesterol and fatty acids are reesterified to form triglycerides. After the addition of small amounts of protein, cholesterol, cholesterol ester and phospholipid, the reconstituted triglycerides are packaged into predominantly prechylomicron particles and transported to the basolateral membrane. At the basolateral membrane, the particles are secreted by exocytosis and released into the intestinal lymph in chylomicrons and very low density lipoproteins. In the following section, mechanisms of lipid transport within the enterocyte will be reviewed.

5.7 TRANSPORT OF LIPIDS INSIDE THE ENTEROCYTE

The utilization of lipids within the enterocyte cytosol requires that they be transported through an aqueous medium to the endoplasmic reticulum and mitochondria for further processing before they can be utilized by the cells of the body. As lipids are poorly soluble in aqueous solution, the difficulties involved in uptake of lipids into the cell are continued inside the cell.

In a landmark study in 1972, Ockner et al [35] reported the isolation of a protein of molecular weight 12000 which could bind long chain fatty acids. This protein was identified when Sephadex chromatography of rat jejunal supernatant with [^{14}C] oleic acid showed association of radioactivity to a single protein. They postulated that this protein may account for differences in the absorption of various fatty acids since they established that binding was greater for long chain unsaturated fatty acids than for saturated and medium chain fatty acids. Just as it was involved in cellular transport of fatty acids in the small intestine, they suggested that it might also be involved in the cellular utilization of fatty acids and possibly certain lipid soluble drugs and toxins, in other epithelial and nonepithelial mammalian tissue. In a later study from the same laboratory, Ockner and Manning [36] demonstrated that the above described jejunal FABP shares immunochemical identity with proteins in the fatty acid binding 12000 mol wt fractions of liver, myocardium and adipose tissue supernatant. Quantitatively. more protein was present in the proximal and middle thirds of the jejuno-ileum than in the distal third of the small intestine and duodenum. Intestinal FABP concentration was higher in rats maintained on a high fat diet than on a low fat diet.

Shields et al [37] used monospecific antisera raised against hepatic FABP and gut FABP to study the localization of these proteins in segments of duodenum, jejunum and ileum isolated from 4, 10, 20, 22 and 60 day old rats. Rabbit antisera was raised against these proteins and cryostat and paraffin sections of intestinal segments exposed to the antisera. Immunoperoxidase staining was hence carried out and the staining pattern in the sections analyzed.

Cellular localization of the two different FABPs was the same except that there was more intense staining for hepatic FABP in the apex of cells in the deep villus area. Staining was limited to cell cytoplasm and was most intense in the proximal rather than the distal intestine, in villus rather than crypt cells and in the apical rather than basal segment of enterocytes. No brush border membrane staining was detected. More intense staining of the apical portion of the duodenal villus tip cells was noted in suckling rats as compared to weaning or adult rats. They postulated that changing patterns of protein activity with aging may relate to variations in dietary fat intake, cell proliferation rates, intestinal cellular anatomy and mechanisms of fat absorption. The presence of these proteins throughout the absorptive cell cytoplasm supported a role for them in the transport of fatty acids from the cytoplasmic side of the brush border membrane to their sites of utilization and perhaps a role in promoting esterification of fatty acids.

Thus, cytosolic FABP activity appears to be maximal in the upper villus segments of the proximal small intestine. This finding would correlate with earlier studies by Padykula [38] that, on histological examination of the jejunum following a lipid meal, the apical cytoplasm of absorptive cells in the upper third of the villus were packed with lipid droplets while the cells nearest the crypts were generally free of lipid. However, is the increased activity of FABP in the cells of the upper villus associated with increased uptake from the intestinal lumen of lipids by these cells relative to the uptake by the cells of the lower villus and crypt?

Autoradiographic studies of feline jejunal uptake of PA and OA by Haglund et al [39] demonstrated a gradient of fatty acid absorption along the crypt-villus axis (CVA). In this in vivo study, radiolabelled fatty acid was incubated in isolated segments of proximal jejunum in anaesthetized cats. The animals were subsequently sacrificed and autoradiographs of intestinal epithelial cells obtained. The autoradiographs demonstrated that the presence of both PA and OA predominantly in the cells of the upper villus. During intestinal ischemia induced by cutting off the blood supply to the intestine, this distribution was altered and the lipids were found more evenly distributed along the villus. They postulated that the increased autoradiographic activity in the upper villus was related to a countercurrent exchange which transported absorbed fatty acid from the proximal villus to the villous tip where it was used to construct chylomicrons. Disruption of the villus blood supply disrupted the countercurrent exchange resulting in a more equal appearing lipid uptake along the CVA.

Uptake of glucose has been examined using isolated BBMV harvested from along the CVA [40,41,42]. These studies demonstrated a gradient of glucose uptake by the BBM along the CVA with greater uptake in the villus tip and upper villus than in the lower villus and crypt. Studies have, however, not been carried out to determine whether a gradient of lipid uptake occurs along the CVA. It can be postulated that, as the physical properties and composition of the BBM change along the CVA, lipid uptake would also vary. Results to confirm or refute this idea are contained in the current study.

In summary, following lipid absorption across the UWL and BBM, the hydrophobic lipids must be transported through the cell cytosol to sites in the endoplasmic reticulum and mitochondria where they can be further processed for transport out of the cell and into the body circulation. Transport of fatty acids within the enterocyte is aided by the presence of cytosolic FABPs. Studies have demonstrated that FABPs found in the enterocyte cytosol are similar to that found in the liver, heart and adipose tissue. One intracytoplasmic FABP is a 12000 mol wt molecule which is found in highest concentrations in the proximal jejunum with lesser concentrations in the duodenum and distal small bowel. It is concentrated in apical portions of the enterocyte and its concentration increases in response to a high fat diet.

Concentrations of cytosolic FABP are greater in the upper villus as compared to the lower villus and crypt. It has not yet been established whether a gradient of lipid uptake exists along the CVA. One of the purposes of the current study is to determine whether indeed such a gradient exists.

5.8 CONCLUSION

Lipid absorption is a complex process. Initially, dietary fats undergo emulsification in the stomach and partial enzymatic hydrolysis by lingual lipase. They are then released into the small intestine where they undergo hydrolysis by the pancreatic lipase-colipase complex followed by solubilization of the monoglycerides, fatty acids and cholesterol in mixed bile acid-lipid micelles.

The route by which fatty acids traverse the UWL to be absorbed across the BBM is unclear. Data has been advanced suggesting that lipids leave the mixed micelles to enter the BBM across an aqueous phase in apposition to the BBM. Dissociation of the lipid from the micelle in these circumstances is aided by the presence of an acidic microclimate on the surface of the BBM. However, lipids may dissociate from the micelle as a result of "collisions" between the BBM and the micelle with subsequent absorption of the lipids by the BBM without an intermediate aqueous-lipid phase.

In the case of molecules to which the BBM is particularly permeable, movement across the UWL may be the rate-limiting step in the movement of these molecules into the enterocyte. UWL resistance is particularly important in the movement of long chain fatty acids and cholesterol into the cell.

Absorption of lipids by the BBM is passive at higher concentrations but carrier-mediated at lower concentrations. Once absorbed by the BBM, fatty acids are incorporated into the membrane with subsequent alteration of the membrane protein mediated transport and passive diffusion properties. The mechanisms by which dietary lipids alter membrane function are as yet unclear. They may affect membrane transport properties by altering membrane fluidity or altering the lipid domain of membrane transport proteins.

After they have traversed the brush border membrane, lipids are taken up by cytosolic FABPs which transport the fatty acids, monoglycerides and cholesterol to the endoplasmic reticulum and mitochondria. The fatty acids and monoglycerides are hence reesterified into triglycerides, packaged with proteins into predominantly prechylomicron particles and transported to the basolateral membrane. The lipid rich particles are released into the intestinal lymph from which they reach the liver and eventually all organs of the body.

Lipid uptake along the CVA has yet to be studied. In this study, the question of whether a gradient of fatty acid uptake along the CVA axis will be considered.

5.9 BIBLIOGRAPHY

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CHAPTER 6

EXPERIMENTAL SECTION

6.1 EXPERIMENTAL MATERIALS AND METHODS

6.1.1 Preparation of Tissue

The guiding principles in the care and use of laboratory animals, approved by the Canadian Federation of Biological Societies and by the Council of the American Physiological Society, were observed in the conduct of this study. Mature female New Zealand white rabbits weighing 1.6-2.2 kg were given ad libitum access to standard Purina® rabbit chow and water until the morning of study. Animals were sacrificed by a lethal injection of sodium thiopental (240 mg/kg) into a marginal ear vein. The abdominal cavity was rapidly opened and 80 cm of jejunum distal to the ligament of Trietz was removed. This jejunal segment was gently flushed with 100 ml iced saline and was divided into 20 cm segments. The intestinal mesentery was removed and the jejunal loops were everted over glass rods.

The subsequent steps of enterocyte harvesting were begun within 20 min of sacrificing the rabbits. The two ends of each jejunal segment were tied after filling the segment with isolation buffer I (154 mM NaCl, 10 Mm NaH_2PO_4, 1.5 Mm EDTA, 0.5 Mm glutathione, 0.1 Mm phenylmethylsulfonyl fluoride, pH 7.3). Segments of jejunum were individually suspended in beakers containing 20 ml of citrated buffer solution (96 mM NaCl, 1.5 mM KCl, 8.0 mM KH_2PO_4, 5.6 mM Na_2HPO_4, 27 mM sodium citrate, 0.1 mM phenylmethylsulfonyl fluoride, pH 7.3) and were gently shaken for 15 min in a water bath heated to 37°C. This initial exposure to citrated buffer solution promoted enterocyte dissociation from the underlying lamina propria. Jejunal segments were then transferred to beakers containing 20 ml of isolation buffer. The initial solutions, labelled fraction 1 (F1), were pooled in 250 ml polypropylene bottles and stored on ice. After 40 min of agitation, the resultant solutions were labelled fraction 2 (F2) and pooled in 250-ml polypropylene bottles for further processing.

Next, the intestinal segments were transferred to beakers containing 20 ml isolation buffer, and were agitated for 10 min. Solutions from this step were pooled as fraction 3 (F3) and the segments were transferred to a fourth series of beakers containing isolation buffer. After 25 min of agitation, incubation solutions were recovered, labelled fraction 4 (F4) and pooled in cooled 250-ml polypropylene bottles.

Intestinal segments were transferred to a final series of beakers for 10 min of agitation. The resultant solutions were labelled fraction 5 (F5) and pooled in similar fashion as the previous collected fractions. The intestinal segments were then opened longitudinally and the remaining mucosa was removed by gently scraping with a microscope slide. Mucosal scrapings were pooled in cooled 250-ml polypropylene bottles containing 160 ml of isolation buffer, labelled as fraction 6 (F6) and saved for further processing.

In order to produce more distinct populations of enterocytes from along the CVA, enterocytes from F1, F3 and F5 were not utilized in the study of nutrient uptake along the CVA and therefore BBMV were not prepared from these fractions. All six fractions were utilized in histology and enzyme assay studies to validate the technique of enterocyte fractionation along the CVA.

All subsequent steps were carried out at a temperature of 4°C. Each fraction (F2, F4, F6) was treated separately in similar fashion. Fractions were centrifuged in a Beckman JA-14 rotor (Beckman Instruments Inc. Palo Alto CA) at 2400 g for 5 min. The resultant supernatant was discarded and the pellets were resuspended in 115 ml of mannitol-Tris buffer (300 mM D-mannitol - 10 mM Tris-HCL, pH 7.0) in 250-ml polypropylene bottles. Fractions were then homogenized using a Brinkmann Polytron® (Brinkmann Instruments, Westbury, NY) at a setting of "6" for 10 sec, and were centrifuged at 600 g in a Beckman JA-14 rotor for 15 min. Homogenates were discarded and $CaCl_2$ was added to the supernatants to yield final concentrations of 10 mM $CaCl_2$. The resultant solutions were stirred on ice for 10 min to precipitate subcellular components, and were then centrifuged at 7,800 g for 20 min. The pellets were discarded and the supernatants were centrifuged at 30,000 g for 20 min. Following this, supernatants were discarded and the pellets were resuspended in 20 ml of isolation buffer II (300 mM D-mannitol - 10 mM Hepes-Tris, pH 7.4). The resultant solutions were homogenized using a Brinkmann Polytron® at a setting of "6" for 15 sec, and were then centrifuged at 30,000 g for a final 20 min. The supernatant was discarded and the pellet was resuspended in 1.0-1.5 ml of Isolation Buffer II. Aliquots were taken for protein determination by a modification of the Lowry method using bovine serum albumen [1,2], and for DNA determination by a modification of the Dische reaction of DNA with diphenylamine [3,4].

Sections 1 cm in length were taken from jejunal segments at the end of each step in the fractionation process. These specimens were fixed in formaldehyde solution, embedded in paraffin wax, sectioned for light microscopy parallel to the CVA, and stained with hematoxylin and eosin. Photomicrographs were obtained of each specimen to determine villus height in each fraction as well as the depth of mucosal scraping in F6.

Photomicrographs of histological sections of segments of jejunum demonstrated a progressive decline in the height of villi with each successive fractionation step, with F2 demonstrating removal only of the upper villus enterocytes and F4 demonstrating removal of the upper villus and mid villus enterocytes. Histology of F6 showed virtual absence of villi (data not shown).

6.1.2 Enzyme Activity Determination

Enterocyte homogenates and BBMV were obtained from along the CVA, as described above in Section 1. Enzyme activity was assayed in both enterocyte cell homogenate specimens and in the BBMV enriched suspensions. Alkaline phosphatase (ALP) activity was measured by incubating the study sample with 2-amino-2-methyl-1-propanol buffer in the presence of $MgCl_2$ at pH 10.25. With the addition of p-nitrophenyl phosphate, p-nitrophenol was formed as a result of ALP-induced hydrolysis, and the solution changed colour. Photometric determination of the resultant colour change allowed a quantification of ALP activity in each specimen. Invertase (INV) activity was measured by incubation of the sample with sucrose with assay by a glucose-specific hexokinase reaction of the glucose formed.

Enrichment of enzyme activity in BBMV suspensions as compared to cell homogenates was demonstrated by ratios of 4.1 for INV activity in F2, 6.1 for F4 and 5.1 for F6, and by ratios of ALP activity of 2.6 for F2, 4.0 for F4 and 5.3 for F6. This data is shown in Table 1.

To indirectly assess thymidine kinase (TK) activity, rabbits were injected intravenously with 400 μCi of [3H]-methyl thymidine (86 Ci/mmol, Amersham International, Amersham, UK) in 0.9% NaCl solution, and were sacrificed two hours later. Enterocytes were isolated from along the CVA, cell homogenates were prepared from each fraction, and were transferred to scintillation counting vials (20 ml Fisher Scientific, Montreal, Canada). Scintillation cocktail (Ready-Safe Liquid Scintillation Cocktail, Beckman Instruments Inc. Fullerton, CA) was added and specific activity of [^{3}H]-methyl thymidine was determined for each fraction.

6.1.3 D-Glucose Uptake

To determine uptake as a function of time of incubation, a solution of 100 μM D-glucose was made up in D-glucose transport buffer (100 mM NaSCN - 1 mM $MgCl_2$ - 2 mM $CaCl_2{\cdot}H_2O$ - 100 mM D-mannitol - 10 mM Hepes-Tris, pH 7.4). D-glucose in this solution consisted of 1% D-[^3H] D-glucose (4.4 Ci/mmol, Amersham International, Amersham, UK) which had been dried down under nitrogen and 99% reagent grade D-glucose. The selected times of incubation were 5, 10, 15, 30 and 45 sec and 1, 1.5, 2, 3, 5, 10 and 15 min.

BBMV uptake of substrate was studied at room temperature (20·C). The vesicles had been initially suspended in Na^+-free solution so that a 100 mM inwardly-directed Na^+ gradient was present at the start of incubation. Incubations of under 1.0 min were carried out by mixing 20 μl of vesicle suspension with 20 μl of D-glucose solution in 17 X 100 mm polystyrene test tubes (Fisher Scientific, Montreal, Canada). For time trials of 1.0 min and longer, incubations of 50 μl of both vesicle suspension and D-glucose solutions were used in similar polystyrene tubes. Reactions were initiated by mixing of the solutions with a vortex mixer. Uptake was terminated with the use of a stopping buffer (100 mM NaSCN - 0.6 mM phlorizin - 0.15 M KCl - 2 mM Hepes-Tris, pH 7.4). Aliquots of the subsequent solution were rapidly filtered through a 0.45 μ nitrocellulose filter (Micron Separations Inc. Westboro, MA) under vacuum on an Amicon Filtration Manifold VFMI. Filters were prewashed with stopping buffer. The filters were rinsed with 4 ml stopping buffer and were placed in scintillation counting vials. The filters were air-dried for 10 minutes at 55·C, and 7.0 ml of scintillation cocktail was added to the scintillation vial. Radioactivity associated with the filter was measured using a Beckman LS 9800 liquid scintillation counter (Beckman Instruments, Inc., Irvine, CA).

The value of radioactive uptake for each sample was the net radioactivity after subtraction of the nonspecific binding of radioactivity to the filter, as determined by measuring the uptake of radioactive substrate by the filter in the absence of vesicle suspension.

D-glucose uptake trials were carried out in identical fashion for vesicle suspensions derived from F2 (upper villus), F4 (mid villus) and F6 (lower villus/crypt). Each data point represents the average of four duplicate studies each using a minimum of four rabbits.

The time-course study of D-glucose uptake for each fraction showed an "overshoot" phenomenon, with initial linear rates of uptake to 15 sec. Accordingly, a 5 sec duration of incubation was considered representative of substrate uptake at "initial rate" and was selected for the subsequent studies of D-glucose uptake by the BBMV at varying D-glucose concentrations.

BBMV uptake as a function of D-glucose concentration was studied with D-glucose concentrations varied from 50 to 1000 μM at increments of 50 μM. Studies were carried out in an equivalent fashion for BBMV suspensions derived from each fractions. Each data point in this section represents an average of six duplicate studies from a minimum of four rabbits.

6.1.4 Linoleic Acid Uptake

The uptake of linoleic acid (LA) by jejunal BBMV was studied in a similar fashion as D-glucose uptake: 100 μM LA solutions were suspended in lipid transport buffer (1 mM $MgCl_2$ - 2 mM $CaCl_2{\cdot}2H_20$, 100 mM D-mannitol - 10 mM Hepes-Tris - 2 mM taurocholic acid, pH 7.4). LA in this solution consisted of 20%[^{14}C] LA (55.6 mCi/mmol Amersham International, Amersham, UK) and 80% reagent grade LA. In the preparation of the LA, a 600 μM unlabelled LA stock solution was prepared by dissolving the required amount of LA in 25 ml chloroform. Aliquots were pipetted into tubes, dried under nitrogen and stored at -80·C. On the day of experimentation, the appropriate amount of unlabelled LA was reconstituted in chloroform with addition of the required amount of radiolabelled LA. The labelled and unlabelled LA was hence dried down under nitrogen and reconstituted in lipid transport buffer.

Studies were carried out for 5, 10, 15, 30 and 45 sec, and for 1, 1.5, 2, 3, 5, 10 and 15 min. Uptake was arrested using stopping buffer (0.2 mM phloretin - 0.15 M KCl - 2 mM Hepes-Tris, pH 7.4]. To prevent the filters from binding LA, the filters were prewashed with 2.0 ml of cold wash solution [2 mM TC - 500 μM LA - 0.15 M KCl, 2 mM Hepes-Tris]. Otherwise, similar filters were used, as described above, for the studies of D-glucose uptake.

To determine LA uptake as a function of concentration, an incubation time of 5 sec was chosen. LA concentrations of 25-200 μM were used. Each data point of LA uptake represents the average of six duplicate studies from a minimum of four rabbits.

6.1.5 Expression of Results and Statistical Analysis

Glucose uptake kinetics were determined by non-linear regression analysis (Michaelis-Menten) using the Enzfitter® software package (Biosoft), and by the Systat[1] program for best-fit curves. The analysis was performed by fitting the observed data points to the Michaelis-Menten equations. As the variance increased with the size of the variable Y (rate of uptake of glucose or LA), data points were weighted in proportion to the reciprocal of the within concentration estimates of variance. Nonlinear regression analysis was estimated for each treatment. The iterative analysis employed sought to minimize the residual sum of squares between the observed and predicted values. Convergence occurred with 20 iterations using the nonlinear regression modules of Systat. The values of V_{max} and K_m were also assessed by linear regression analysis of the Lineweaver-Burke plot (inverse of glucose uptake versus inverse of glucose concentration), and the Eadie-Hofstee plot (D-glucose uptake versus uptake/glucose concentration).

All values given in the Tables and Figures represent the mean ± standard error. ANOVA analysis and Student Newman-Keul's test were used to search for differences between means, with 0.05 taken as the upper limit of significance.

The rates of uptake of glucose and LA at initial rates were expressed as $pmol^{-1} \cdot mg\ protein^{-1} \cdot 5sec^{-1}$ and $pmol \cdot unit$ BBMV surface $area^{-1} \cdot 5sec^{-1}$·, and $nmol \cdot mg\ protein^{-1} \cdot 5\ sec^{-1}$ and $nmol \cdot unit$ BBMV surface $area^{-1} \cdot 5\ sec^{-1}$, respectively. The minimum surface area of the BBMV was calculated from the equilibrium value of glucose uptake during incubations of varying times between 0 and 15 minutes.

6.2 RESULTS

6.2.1 Validation of Techniques

The ALP and INV activity as well as the protein and DNA content were measured in each fraction. Thus, the ALP and INV activity per g protein and per mg DNA content of each fraction could be calculated. Uptake of [^{3}H] methyl thymidine was determined as radioactive decays per min (dpm), and was similarly determined per mg protein and µg DNA content of each fraction. Uptake of [^{3}H] Methyl Thymidine by the enterocyte fraction was assumed to be representative of underlying TK activity in that fraction. The volume of solution for each fraction was measured and the total content of protein and DNA per fraction determined. Subsequently, the total amount of protein and DNA obtained from along the CVA in the six fractions was determined, and the percentage of protein or of DNA in each fraction relative to the total amount obtained was calculated. The relative position of each fraction on a continuum of protein and DNA content along the CVA could hence be determined.

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[1] SYSTAT Inc., Illinois, 1800 Sherman Avenue, Evanston, Illinois 60201

The cumulative activity of each enzyme in all six fractions was calculated, and the percentage of total enzyme activity in each fraction was determined. Enzyme activity could thus be analyzed in several ways and expressed in each fraction relative to the protein or DNA content of that fraction or as percentages of the total amount of enzyme, protein and DNA harvested. Initially collected fractions were designated as early percentages, with subsequent fractions being added to previous fractions. F2 represented the point at which 6 to 18% of total protein and DNA had been collected, while F4 was located at the point at which 30 to 45% of protein and DNA had been collected. F6 represented the point at which 100% of protein and DNA had been collected.

ALP activity was lower in enterocyte fractions enriched in mid-villus and lower villus/crypt cells, as compared to fractions enriched in upper villus cells; this was observed regardless of whether ALP activity was expressed relative to the protein or DNA content of the sample (Figures 1A and 1B). When ALP activity was expressed per g of protein, the highest activity was in fraction 1, with intermediate values in F3 and F4 and the lowest values in F6 (Figure 1A). When ALP activity was expressed per mg DNA, there was a gradual increase in activity between F1 and F3 with a subsequent decline in activity (Figure 1B). When the percentage of total ALP activity was determined for each fraction, activity was greatest in the final three fractions (Figure 1C). However, when activity was calculated per g protein and per mg DNA and was expressed relative to the % of total protein or DNA harvested to that point along the CVA, activity was greatest in the initial fractions and progressively declined as the % of total protein and DNA obtained from along the CVA increased (Figure 1D and 1E). Irrespective of whether ALP activity was expressed per g protein or per mg DNA, there was a progressive increase in the cumulative percentage of total activity along the continuum of protein and DNA obtained from along the CVA (Figure 1F and 1G).

When INV activity was expressed relative to the protein or DNA content of each of the six fractions, activity peaked in the middle fractions with lesser values in the earliest and later fractions. Activity was lowest in the final collected fractions (Figures 2A and 2B). Expressed per mg protein, INV activity was greatest in F3; when expressed per mg DNA, the greatest activity of INV was in F2 (Figure 2A and 2B). Similar to ALP, the percentage of total INV activity was greatest in the final three fractions (Figure 2C). When INV activity was expressed either per g protein or per mg DNA, the greatest activity was seen at the point at which between 20 and 40% of the protein and DNA had been harvested, corresponding to the second to third fractions (Figure 2D and 2E). When activity was expressed per g protein and per mg DNA, there was a progressive increase in the percentage of total cumulative INV (Figure 2F and 2G).

Enrichment of INV activity in BBMV suspensions as compared to cell homogenates was 4.1 for F2, 6.1 for F4 and 5.1 for F6, whereas enrichment of ALP activity was 2.6, 4.0 and 5.3 for F2, F3 and F6, respectively (Table 1).

The uptake of [^{3}H] methyl thymidine in initially collected fractions was substantially less than in later collected fractions. Over 90% of total uptake occurred in the final collected fraction (Figures 3B and 4B). When uptake was expressed as dpm/mg protein or µg DNA, uptake was highest in F6. However, a second but lower peak of [^{3}H] methyl thymidine uptake was noted in F1 when uptake was expressed relative to protein and in F2 when uptake was expressed relative to DNA (Figure 3A and 4A). [^{3}H] methyl thymidine uptake, when expressed relative to % total protein recovered demonstrated that maximal uptake occurred in the final fraction with a second lesser peak in the initial (less than 20% total protein) fractions (Figure 3C). When uptake was expressed relative to total DNA recovered, uptake similarly was greatest in F6, with a second lesser peak found in the initial (less than 20% total DNA) fractions (Figure 4C).

The amount of DNA present per cell remains constant as that cell matures along the CVA. However, cellular morphology changes along the CVA. It might therefore be anticipated that the protein content of the cell changes along the CVA and thus the ratio between protein and DNA in each cellular fraction might also vary along the CVA.

Determination of fraction protein content versus DNA content demonstrated that the amount of DNA relative to the amount of protein present did vary along the CVA. The lowest ratio of protein to

DNA content was seen in the initial fractions, with the ratio reaching its highest value in the middle fractions and declining in the later fractions (Figure 5). This suggests that the relation between cellular DNA and protein content is not constant along the CVA. Thus, data expressed in relation to protein content of a fraction cannot be directly compared to data expressed in relation to DNA content.

6.2.2 Uptake Studies

Validation studies confirmed that the current technique was able to isolate fractions of enterocytes from along the CVA. F2 contained enterocytes originating from the upper villus while F4 contained enterocytes from the middle villus and F6 contained enterocytes from the lower villus and the crypt. Subsequent studies of nutrient uptake were carried out using BBMV from these three fractions.

a) Glucose

The Na^+-dependent uptake of 100 μM D-glucose into BBMV isolated from F2, F4 and F6 demonstrated a time-course "overshoot", with initial rates of uptake being linear up to 15 sec (Figure 6). Peak glucose uptake was almost twice as high in fractions enriched in mid-villus and crypt enterocytes (F4 and F6) as in the fraction enriched in upper villus enterocytes (F2).

An equilibrium state was achieved within 15 min of onset of incubation of BBMV in D-glucose containing solution (Figure 6). At equilibrium, the concentration of D-glucose in the intravesicular space is equal to the D-glucose concentration in the external solution. Since the intravesicular space is substantially smaller than the extravesicular space, the concentration of D-glucose on both sides of the vesicle wall following equilibrium will not be significantly less than the concentration of D-glucose in the external solution at the beginning of the time trial. Thus, at equilibrium, the concentration of D-glucose in the intravesicular space will be very close to this value. The uptake of D-glucose by the vesicle solution at equilibrium was: F2; 275 pmol/mg protein; F4; 487 pmol/mg protein; and F6; 655 pmol/mg protein. The calculated intravesicular volumes of the vesicles in F2 is 2.75 μL/mg protein while that of F4 is 4.87 μL/mg protein and that of F6 is 6.55 μL/mg protein. We chose to use these values to calculate the surface area of the BBMV, and to express glucose and linoleic acid uptake on this basis.

As BBMV form spheres in solution, the total volume of the BBM vesicles can be expressed by the formula $4/3 \cdot \pi R^3$, where R equals the radius of the sphere. The calculated values of R would therefore be: F2 0.869μm, F4 1.052 μm and F6 1.161 μm. The minimum surface area of the BBMV sphere is expressed by the formula $4 \cdot \pi R^2$. The calculated surface area of the BBMV are: F2 9.49 μm^2/mg protein, F4 13.89 μm^2/mg protein and F6 16.93 μm^2/mg protein and the ratios of the surface areas of the BBMV with the surface area of F2 vesicles assuming the value of 1.0 would be: F2 1.0, F4 1.46 and F6 1.78. Thus, for every unit of surface area in F2 that contains one unit of protein, F4 contains 0.68 units and F6 contains 0.56 units.

Varying D-glucose concentrations from 50 to 1000 μM and using periods of incubation of 5 sec demonstrated a curvilinear relationship between D-glucose concentration and uptake (Figure 7). The uptake of glucose was similar in all fractions, up to a glucose concentration of 400 μM. At higher concentrations, uptake of glucose was lower in F2 than in F4 or F6. Since the effective resistance of the intestinal UWL is negligible for isolated cells or BBMV [5], the value of the apparent Michaelis affinity constant was taken as the value of the true constant, K_m. The absolute values of the K_m and V_{max} varied depending upon the method used to estimate the kinetic constants (Table 2).

Several methods were used to estimate the values of V_{max} and K_m; Systat; Enzfitter with simple weighting, with proportional weighting or with proportional plus robust weighting; Sigmaplot; and the linear transformation plots, Lineweaver-Burke and Eadie-Hofstee. The value of the V_{max} was F2<F6<F4 using Systat, Enzfitter with proportional and proportional plus robust weighting, Sigmaplot and Eadie Hofstee plot, whereas the relative ratios were F6<F2<F4 for Lineweaver Burke plot and Enzfitter with simple weighting (Table 2). Thus, it is clear that the highest value for rabbit jejunum V_{max} for glucose transport is in the mid villus fraction. When the value of V_{max} was expressed on the basis of the estimated BBMV surface area, the highest values were once again for the middle portion of the villus (Table 3). In all methods of analysis the value of the K_m was lowest in F2, but the relative values of K_m in F4 and F6 varied between method of estimation.

b) Linoleic Acid

No overshoot was noted for the rate of uptake of 100 μM LA in F2, F4 and F6, with a plateau of the rate of uptake occurring beyond 5 min incubation (Figure 8). With a 5 sec incubation and solubilization of LA in 2 mM taurocholic acid, a linear relationship was noted between LA concentration and uptake in F2, F4 and F6 (Figure 9A).

The slope of the linear relationship between LA concentration (25-200 μM) and uptake represents the passive permeability coefficient (Pd) of the BBMV. This value was similar for the three fractions obtained from enterocytes along the CVA and was 0.172 $\text{nmol}\cdot 5\ \text{sec}^{-1}\cdot \text{mg protein}^{-1}\cdot \mu M^{-1}$. When LA uptake was expressed on the basis of the calculated BBMV surface area, the Pd was higher for F2 than for F4, and for F4 than for F6 (Figure 9B) with values of 0.185, 0.130 and 0.079 $\text{nmol}\cdot 5\ \text{sec}^{-1}\cdot \text{unit surface area}^{-1}\cdot \mu M^{-1}$, respectively, for F2, F4 and F6.

Although the relationship between BBMV LA uptake and concentration in the incubation medium was linear suggesting passive uptake of LA by the BBMV, the vertical intercept for each fraction was significantly ($p<0.05$) greater than zero.

6.3 DISCUSSION

6.3.1 Isolation Method

Enterocytes can be isolated sequentially from along the CVA and their origin established by determining their profile of enzyme activities [6-16]. Cells in the intestinal crypt are rich in enzymes involved in protein synthesis, whereas in the mid- and upper-villus the major cell functions include the digestion, absorption and metabolism of luminal nutrients. This change in function is reflected by decreasing incorporation of [^{3}H]methyl thymidine as the enterocytes advance up the CVA, with corresponding increases in the activities of INV and ALP (Figures 1 and 2).

Using a technique that involved assaying enzyme activity in segments of intestinal wall horizontally sectioned from along the CVA in adult Wistar rats, Nõrdstrom [13] demonstrated highest values for ALP activity from the upper quarter of the villus with values then declining towards the base of the villus. INV activity was also low closer to the villus base, but persists over a greater length of the villus. Sundaram et al [10] reported ALP activity as a percentage of the values in the villus tip component. This was the highest value, and the activity declined moving from the villus tip to the base of the villus. Freeman et al [15] also found the highest ALP activities to be in the villus tip fraction, with values declining progressively towards the mid-portion of the villus, with little further change between that point and the base of the villus. ALP and INV activities were expressed as $\text{mmol}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$ and plotted against the percent of isolated cells. There was a progressive and similar decline in activity moving from the initial 20% of isolated cells to the final 100% of isolated cells. Morrill et al [11] plotted sucrase/isomaltase activity relative to protein or DNA content, as well as plotting enzyme activity versus percentage of total protein over percentage of total DNA. They found that the highest values appeared in the mid-portion of the CVA when expressed as units/mg protein versus percentage of total protein, whereas sucrase/isomaltase activity was highest in the villus tip when expressed as units/mg DNA or versus percentage of total DNA. This was presumably because the ratio of protein/DNA declined markedly moving from the villus tip to the crypt. Hartman et al [9] expressed sucrase activity as units/gm protein and determined that the highest activity was in the mid-villus fraction, with lower values at the villus tip and lower villus/crypt. Dudeja [8] demonstrated highest values of sucrase when expressed on the basis of protein, in villus tip cells as compared with low values in lower villus and intermediate values in the mid-villus. Thus, the pattern of distribution of ALP and INV activity varies from method to method, and with the manner used to express the results, but activities are generally higher in the upper than in the lower portion of the villus.

In our study, ALP activity progressively fell from F1 to F6 when activity was expressed on the basis of mg protein or μg DNA content of each fraction (Figure 1). Expressed similarly, INV activity was highest in the mid-villus fractions (Figure 2).

Regardless of the method of expression of TK, in previously published studies [8,14,15], greater than 60% of activity appears in the lower villus fraction. Similarly, in our study, the highest uptake of [^{3}H] methyl thymidine was found in the basal portion of the villus (Figures 3 and 4).

Thus, F2 represents the upper portion of the villus while F6 represents the basal portion of the villus and the crypt cells, and F4 represents the mid-portion of the villus. It is important to note, however, that the pattern of change reported in our study and those reported by others depend upon the method used to express the result. It is likely that our upper, mid and lower villus fractions may have been different from those reported by other authors.

6.3.2 D-Glucose uptake

Glucose entry into enterocytes occurs as the result of Na^+-dependent coupling to a cotransporter in the BBM [8,14,15,17-19]. In this study, glucose transport in the presence of an inwardly- directed Na+ gradient (NaSCN_{out} = 100 mM; NaSCN_{in} = 0) at a D-glucose concentration of 100 μM demonstrated the expected time-course "overshoot" phenomenon in BBMV derived from all three fractions (Figure 6). Incubation of BBMV with glucose in concentrations between 50 and 1000 μM demonstrated saturation kinetics (Figure 7). These results demonstrate qualitative similarities in the vesicle uptake of glucose into the three BBMV fractions.

Utilizing BBMV harvested from along the CVA using fractionation techniques, other investigators have established that a gradient of glucose uptake exists along the CVA of the proximal small intestine. Meddings and co-workers [14] demonstrated in fasted young male New Zealand white rabbits that the highest rates of transport of glucose occurred in BBMV isolated from upper- and mid-villus cells. These fractions are approximately comparable to our F2 and F4. They recorded an affinity constant (K_m) for glucose transport of 200.4 μM for BBMV isolated from cells derived from the upper villus, with values of 242.4 μM and 114.3 μM respectively for BBMV derived from cells harvested from the mid-villus and crypt levels. This is in contrast to our finding of a higher K_m for glucose uptake by BBMV obtained from enterocytes isolated from the lower villus/crypt, F6 (Table 2). Also, Meddings and colleagues [14] used the Systat program to obtain estimates of V_{max} which were highest in cells from the upper villus, intermediate in cells from the mid villus and lowest from the lower villus (47.8, 40.0 and 7.7 nmol·mg protein^{-1}·min^{-1}, respectively). These findings have been supported in autoradiographic studies of *in vivo* glucose studies by Kinter and Wilson [20] and Fedorak et al [21] which demonstrated that most glucose uptake occurred in the enterocytes of the upper villus.

This is in contrast to our results with mature, non-fasted female rabbits, in which V_{max} was lower in the upper villus fraction, regardless of the method used to estimate the value of Vmax when not corrected for BBMV surface area (Table 2).

Dudeja et al [8] used the Lineweaver-Burke method to estimate the value of V_{max} and K_m. They demonstrated, in fasted adult male Lewis rats, a K_m for D-glucose transport of 121 μM for BBMV derived from the villus tip cells, a value of 164 μM for BBMV derived from the mid-villus level and 44 μM for BBMV derived from crypt cells. Values of V_{max} of 904, 1296 and 498 pmol·mg protein^{-1}·4sec^{-1} were recorded in fractions enriched with BBMV from villus tip, mid-villus and crypt cells. Freeman et al [15] used the Michaelis-Menten least squares method to estimate V_{max} and K_m; they also demonstrated a gradient of D-glucose uptake along the CVA of male Sprague-Dawley rats, with V_{max} values of 16.3 and 4.5 nmol·mg protein^{-1}·min^{-1} from higher versus lower portions of the villus, and Km values of 482 and 144 μM from the same positions along the CVA.

Thus, in these three studies using rabbit and rat BBMV, V_{max} and K_m were highest in upper and mid-villus enterocytes, when uptake was expressed on the basis of mg protein and when different methods were used to estimate the values of V_{max} and K_m. This was opposite to the gradient described in this study when our data was expressed as pmol.min^{-1}·mg protein^{-1} (Table 2). This difference in the relative values of the V_{max} cannot be explained by differences in the methods used to estimate the value of these kinetic constants.

With each of the methods used to estimate the value of V_{max} the lowest value was obtained in F2. Similarly, the value for K_m was lowest in F2 for each method of analysis. The relative ratio of the V_{max} or K_m between F2, F4 and F6 varied depending upon the method of analysis. For example, for V_{max} the relative values were F2<F6<F4 using Systat, Enzfitter with proportional and proportional plus robust weighting, Sigmaplot and Eadie-Hofstee plot. In contrast, the relative ratios were F6<F2<F4 for Lineweaver Burke plot and Enzfitter with simple weighting. Differences were also noted in the relative values of the K_m depending upon the method used for these estimations. The K_m values were F2<F6<F4 with Systat, Enzfitter simple and proportional weighting groups as well as the Eadie-Hofstee and Lineweaver-Burke plots. In contrast, the ratio was F2<F4<F6 for Systat, Sigmaplot and Enzfitter proportional and robust analyses. Thus, since it is not possible to determine which of these is the "correct" value for the estimation of V_{max} and K_m, it is suggested that Systat and at least one other program be utilized. Regardless of which methods of kinetic analysis were used, the values of the V_{max} and K_m were either lowest in F2 as compared with F4 and F6, or the value of V_{max} was unchanged and the value of the K_m more than doubled between F2 and F6. Thus, different methods of kinetic analysis are unlikely to have been the explanation for our inability to confirm the qualitative nature of the previously-reported gradient of kinetic constants for glucose uptake along the CVA of the jejunum.

One possible explanation for these variations would be to suggest that methodological considerations gave rise to a different population of enterocytes harvested from along the CVA. For example, intestinal scraping to obtain crypt cells was not employed in the studies of Dudeja et al [8] or Freeman et al [15]. Our upper villus fraction used to determine glucose uptake (F2) may have had different transport properties than the very most distal cells sometimes used by others. Other differences between these and our studies included the age or gender of the animals used, and variations in their recent intake of food. In this study, mature female rabbits were used with weights in the range of 1.6 to 2.2 kg, as compared to younger male rabbits with a weight range of 0.5 to 0.75 kg used in the studies of Meddings et al [14]. In a male Wistar rat model, Na^+-dependent D-glucose transport varied with age with young animals having a greater capacity for Na^+-dependent D-glucose transport [22]. The male rats studied by Dudeja et al [8] were fasted for 18 hours prior to sacrifice. Rabbits studied by Meddings et al [14] underwent overnight fasts before being sacrificed. Rabbits used in this study were allowed ad libitum access to food and water up until time of sacrifice. Prior experiments have demonstrated that changes occur in the BBM as a result of malnutrition that may influence transport characteristics [23,24]. It is unknown whether simple fasting overnight without previous protein-energy malnutrition or dietary carbohydrate changes alters the relative values of V_{max} and K_m for glucose uptake along the CVA.

The mechanism of these changes in glucose uptake kinetics along the CVA is unclear. Meddings et al [14] postulated that glucose transport is influenced by BBM fluidity: these workers noted that V_{max} is higher across the rigid BBM of the mid and upper villus enterocytes, as compared to the more fluid BBM of the crypt cells. It is debatable whether these changes in fluidity are the major determinant of D-glucose uptake. For example, Dudeja et al [8] did not demonstrate an association between membrane fluidity and glucose uptake: despite being less fluid than their control counterparts, upper villus BBMV from diabetic rats had similar glucose uptake rates.

The glucose carrier has been cloned (25-29), but it is not yet known whether the lower glucose uptake by the crypt cells is because of a reduction in the amount of carrier or due to the relative inactivity of carrier proteins in the crypt.

Several studies have postulated that more than one D-glucose carrier is present in the BBM. Harig et al [19], using BBMV prepared from human jejunum and ileum, detected a single high-affinity, low capacity transporter along the entire small intestine, plus a second low affinity, high capacity carrier limited to the proximal jejunum. Kaunitz and Wright [17], using bovine and rabbit small intestine preparations, similarly demonstrated two Na^+-dependent saturable transporter systems: a high capacity, low affinity system and a second system with a lower capacity and higher affinity. Malo was able to identify a low affinity high capacity system as well as a high affinity low capacity system in studies of human fetal small intestine [30], but not when using vesicles obtained from human adult tissue donors [31,32]. Both Freeman et al [15] and Dudeja et al [8] have postulated that at least two distinct Na^+-dependent D-glucose transporters are present in the BBM along the CVA. This possibility has been raised previously, based on theoretical considerations [33], and the variations in glucose uptake along the CVA may reflect different proportions of the two postulated carriers, each of which has different kinetic properties. However, molecular biological methods have not suggested the presence of multiple intestinal carriers [25], and Enzfitter analysis of non-parametrically assessed uptake rates is compatible with a single glucose carrier [31,32]. Variations in the ratio of two postulated hexose carriers would not likely explain the qualitative differences in the values of the V_{max} and K_m reported for different CVA fractions in our study, as compared with other authors.

6.3.3 Linoleic acid uptake

The intestinal uptake of lipids has been considered to be by a process of passive diffusion [34]. The unstirred water layer (UWL) and the BBM represent the major barriers to the movement of lipids into the enterocyte. The UWL has been considered the rate-limiting factor in the mucosal uptake of fatty acids [35]. A fatty acid binding protein in the BBM of rat jejunum has been identified, and it has therefore been suggested that the uptake of fatty acids by the BBM may be mediated in part by a membrane fatty acid binding protein [36]. In a study of LA transport by rabbit BBMV, Ling et al [37] demonstrated an "overshoot" phenomenon of uptake as a function of time. We were unable to confirm the presence of an overshoot, either in this study (Figure 8) or in a previous study using a homogenate of enterocytes obtained from along the entire CVA of mature rabbit jejunum [38].

A linear relationship was found between LA concentrations of 25-200 μM and uptake at the initial rate of 5 sec. This is compatible with a process of passive uptake, but does not exclude the possibility of the presence or importance of a membrane fatty acid binding protein. This point is particularly relevant because the Y-axis intercept of the relationship between LA concentration and uptake was different from zero (Figure 9).

Autoradiographic studies with ^{3}H-palmitate have demonstrated higher uptake in villus tip enterocytes than in enterocytes found in the crypt [39]. It is possible that the in vivo uptake of fatty acids varies along the CVA as a result of changes in the effective resistance of the UWL [40]. The fluidity of the BBM falls from crypt to villus tip [14], so that these differences in lipid uptake along the CVA cannot be explained by variations in BBM fluidity. There was no gradient of LA uptake in F2, F4 or F6 (Figure 9A), but when LA uptake is expressed as nmol·unit surface area^{-1}·5 sec^{-1}, the permeability coefficient was greater for F2 than for F4 or F6 (Figure 9B). Thus, lipid uptake in vivo may be greater in the upper portion of the villus because of variations in the dimension of the UWL, greater membrane surface area of the enterocytes from the upper than from the lower portion of the CVA or greater passive permeability coefficients of BBM isolated from enterocytes near the upper portion of the villus.

The intestine is capable of adaptation, with variations in nutrient uptake occurring, for example, with aging [22], dietary lipid changes [41], diabetes mellitus [8,21,42,43], bowel resection [44] or ethanol ingestion [45,46]. In rats rendered hyperglycaemic by the injection of streptozotocin, glucose uptake in the ileum is increased by "recruitment" of enterocytes from more distal locations along the CVA [21]. It remains controversial whether this is the mechanism of increased glucose uptake in the jejunum in diabetes [8,43]. It remains to be established whether altered uptake of lipids in these models of intestinal adaptation results from changes in the passive permeability coefficients of enterocytes on the upper portion of the villus, or from changes in all of the BBM from enterocytes from along the CVA.

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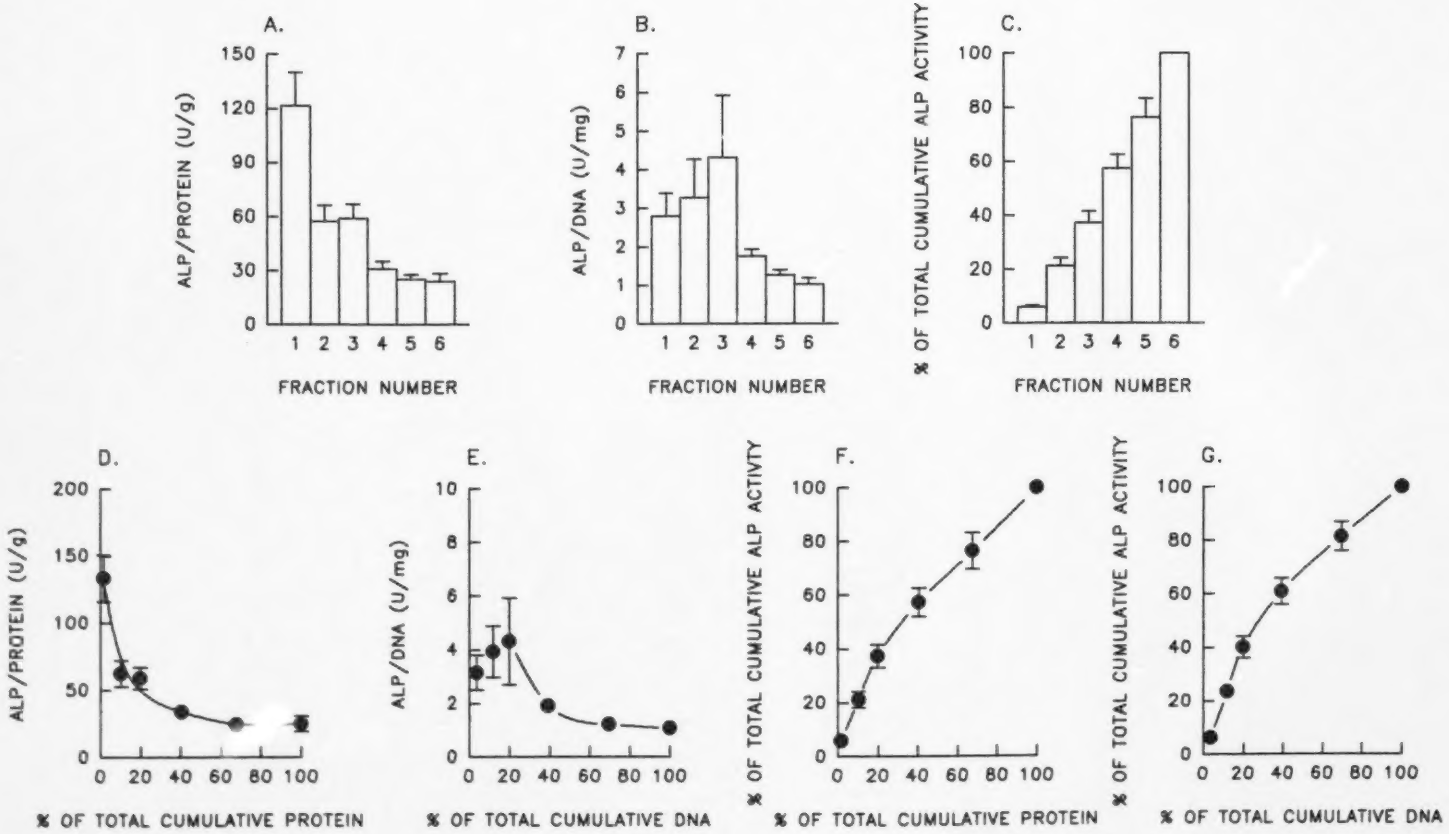

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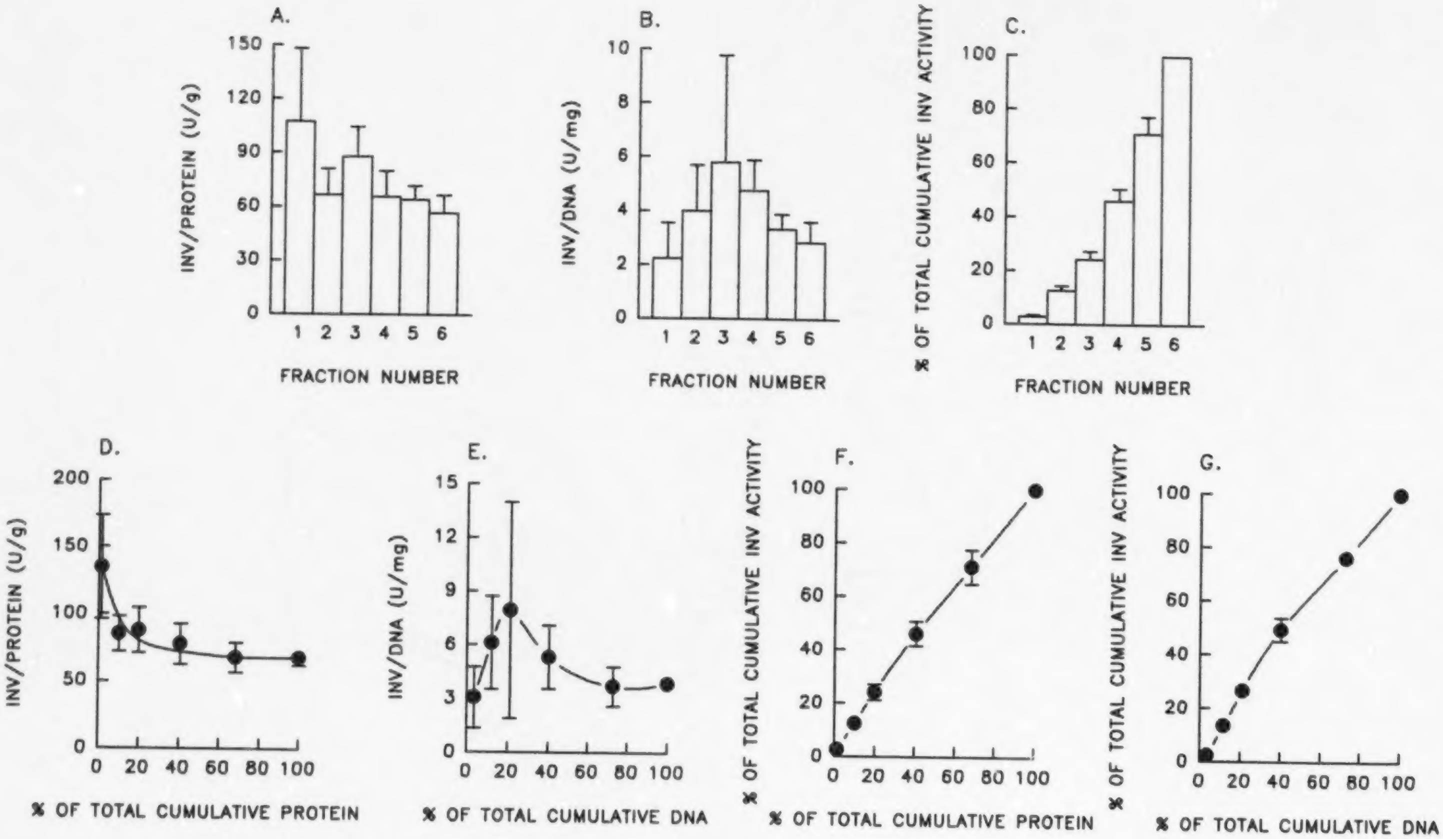

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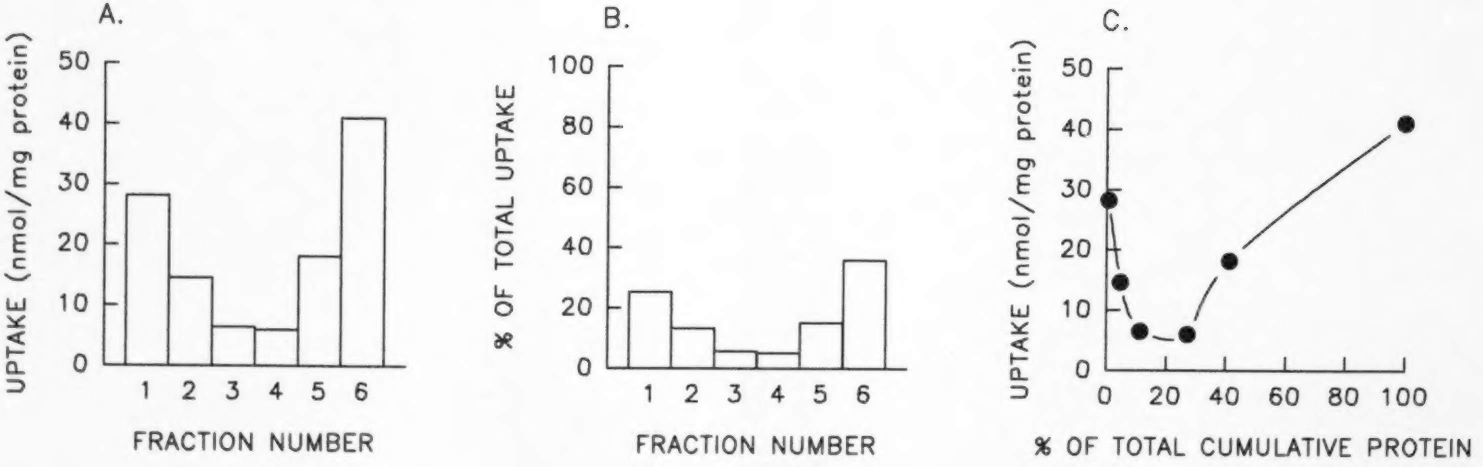

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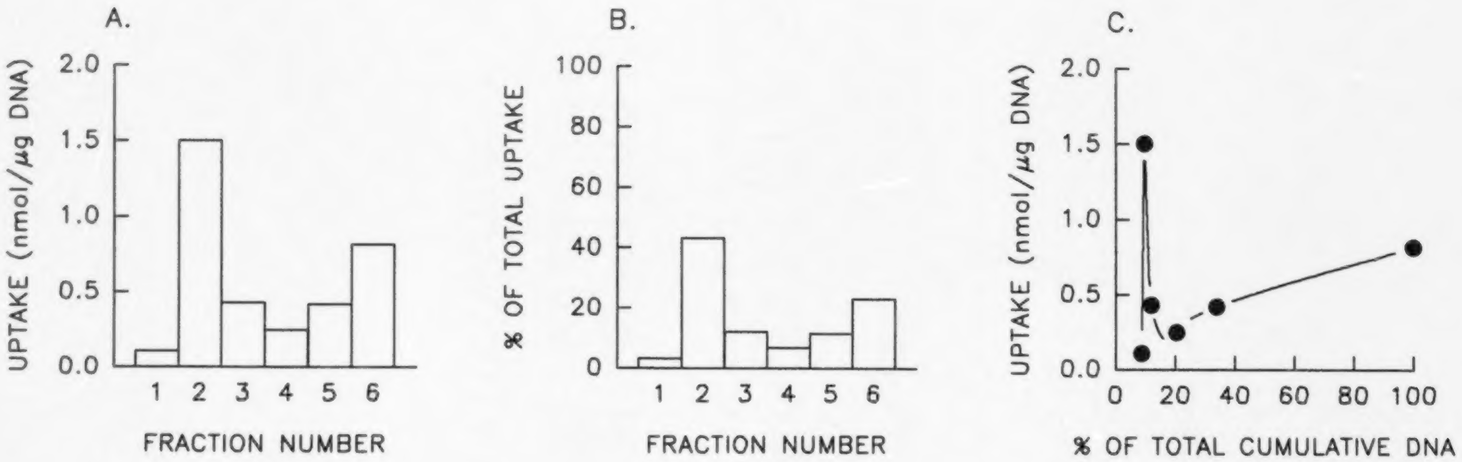

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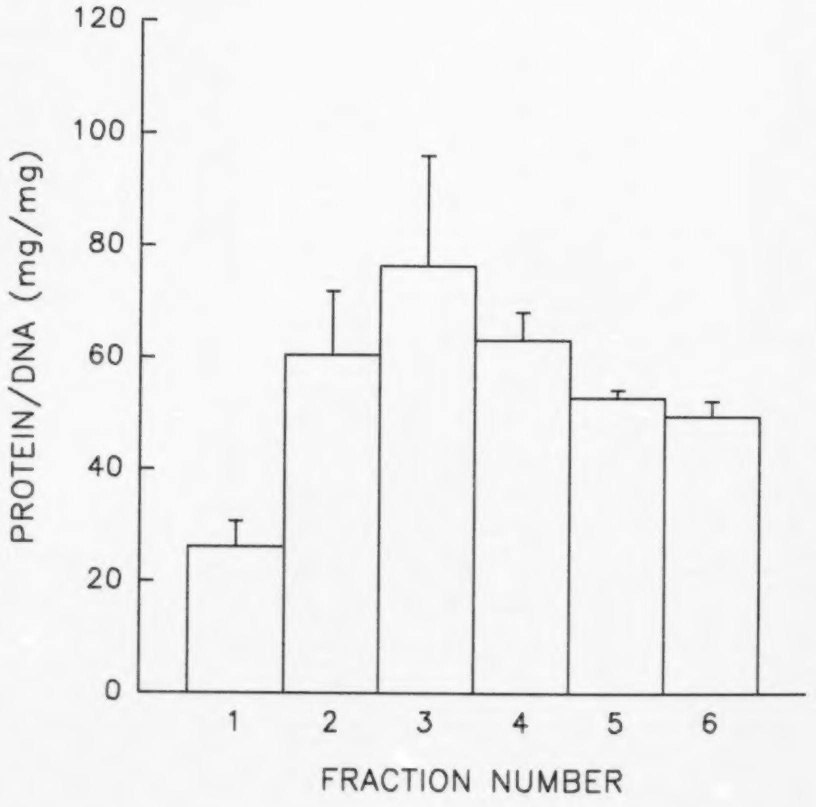

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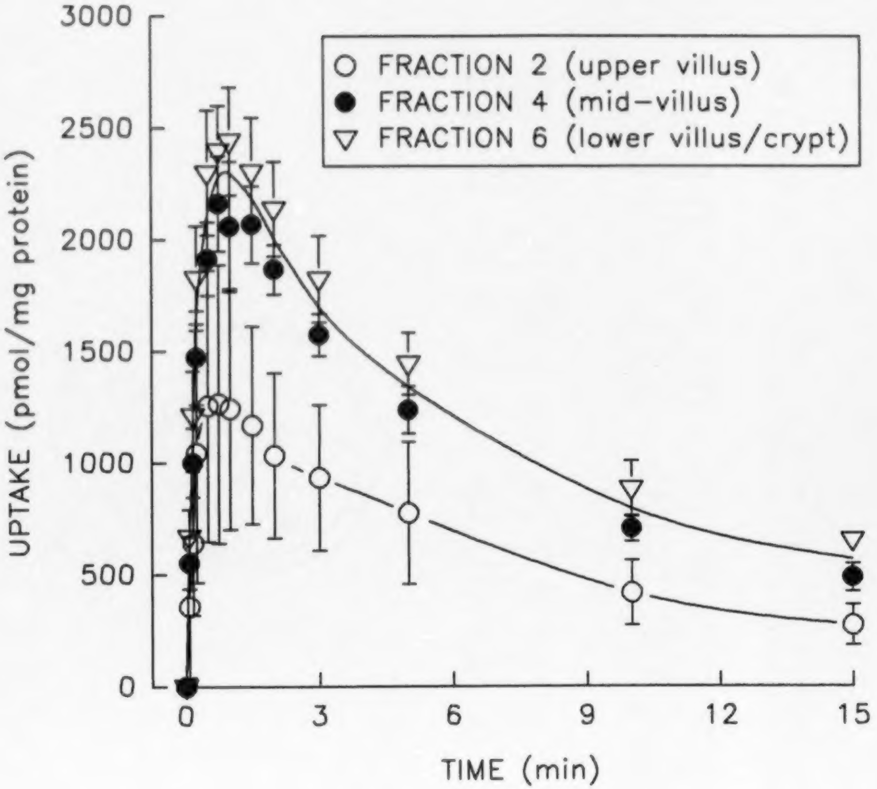

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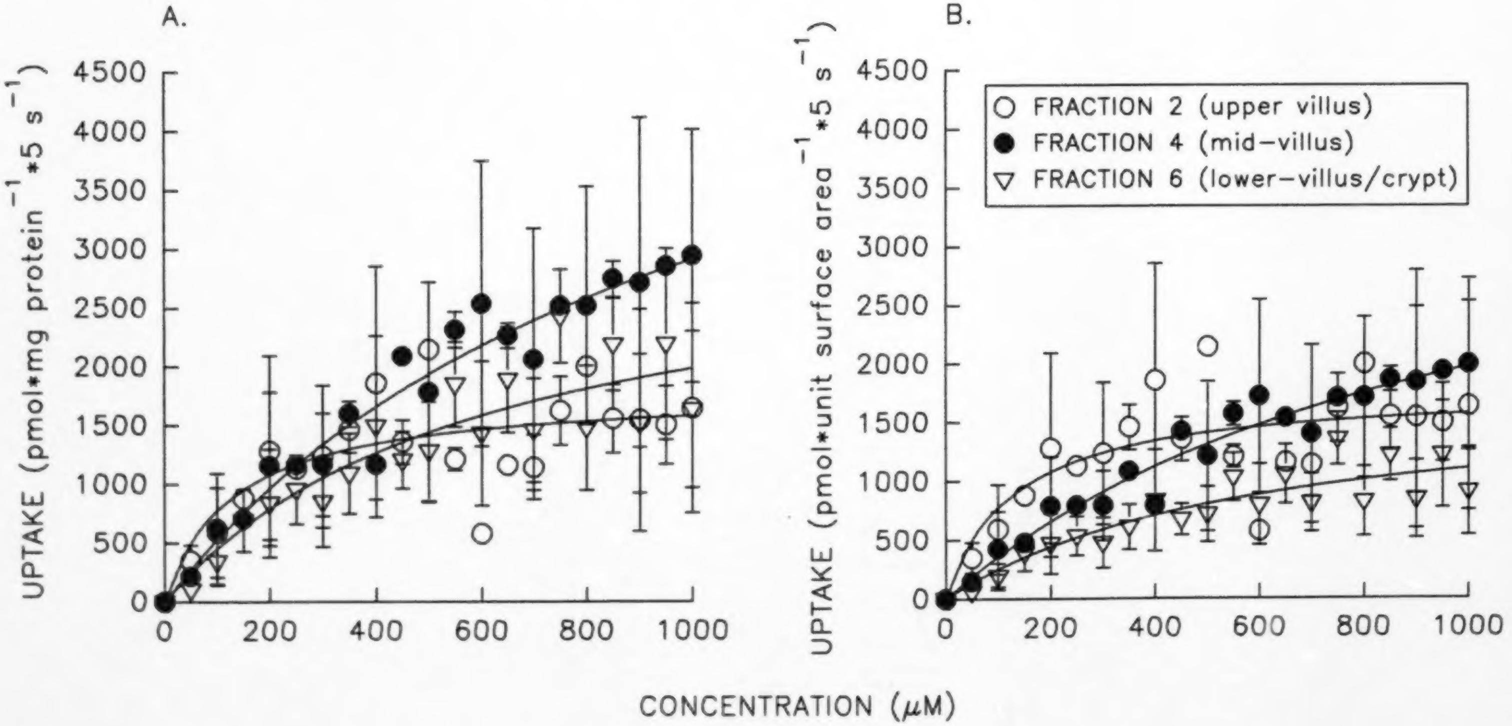

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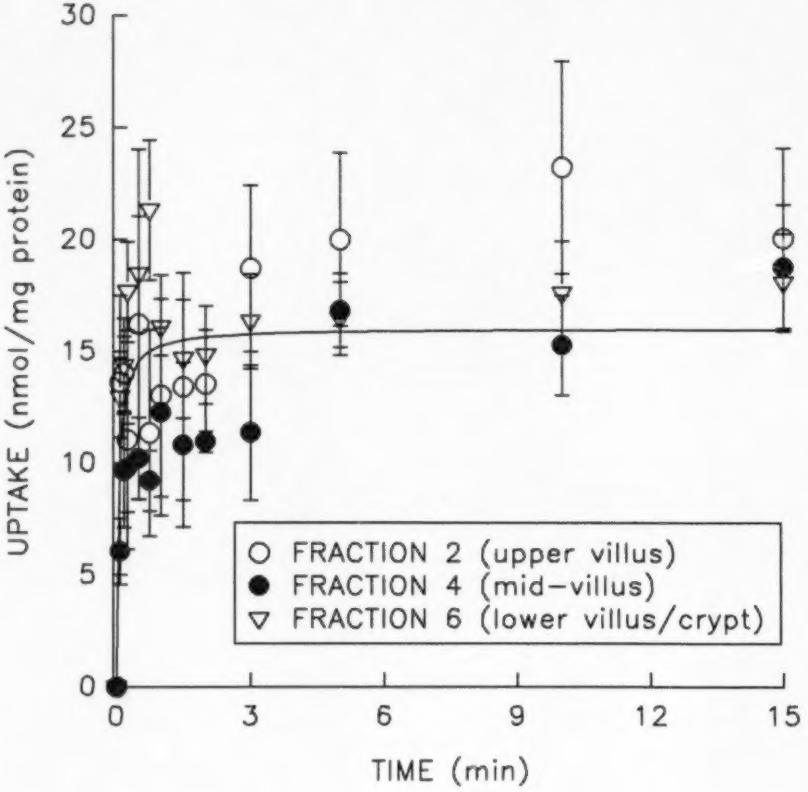

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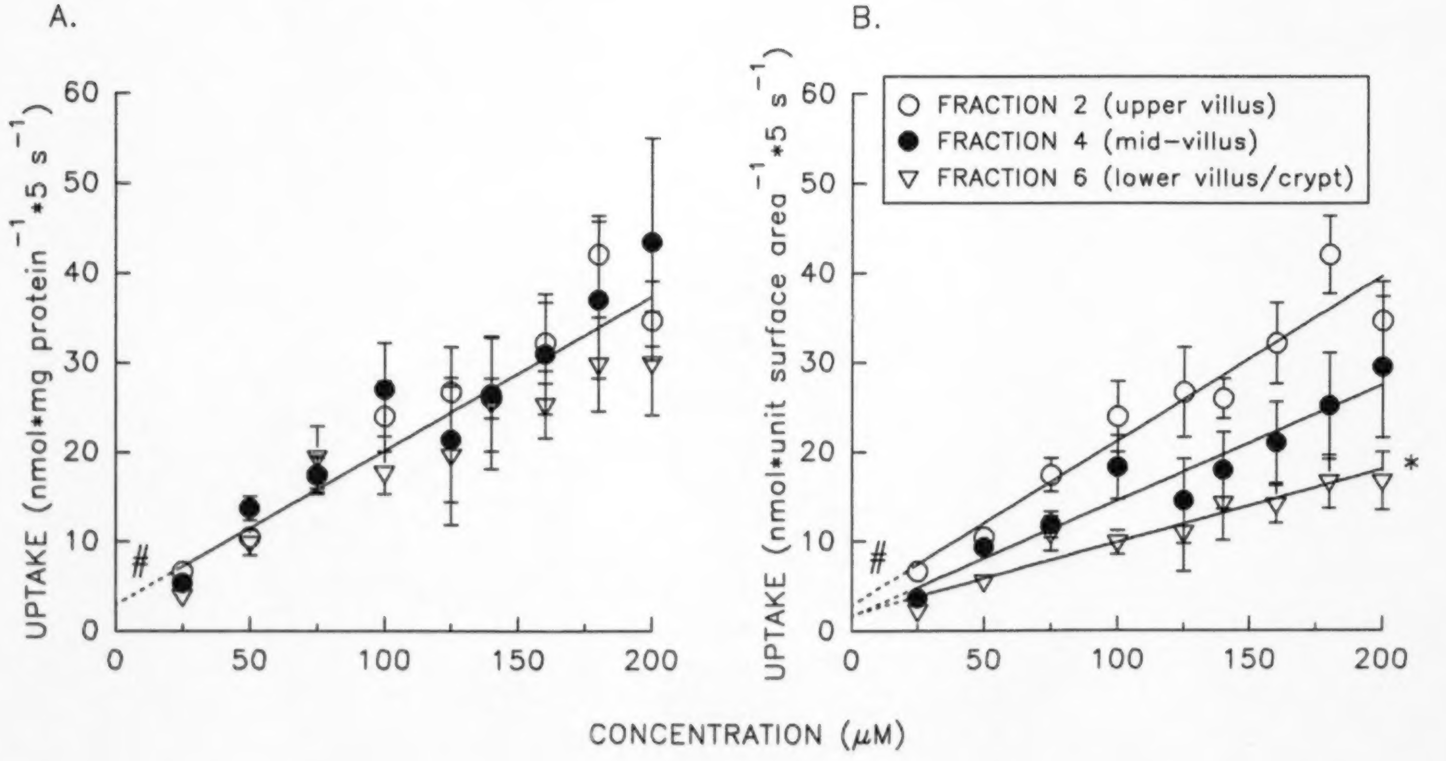

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CHAPTER 7

DIRECTIONS FOR FUTURE RESEARCH

We have established a method for the sequential harvesting of enterocytes from along the crypt-villus axis (CVA) with isolation from these enterocytes of brush border membrane vesicles (BBMV). This has enabled us to confirm the hypothesis that a gradient of linoleic acid uptake exists along the rabbit CVA. By demonstrating a gradient of linoleic acid uptake along the CVA, multiple possibilities for further investigation have been created.

It should now be possible to determine whether the incremental free energy of lipid uptake changes along the CVA. This could be determined by measuring the uptake of short and medium chain fatty acids in BBMV prepared from fractions harvested along the axis. In addition, it should now be possible to quantitatively compare rates of uptake along the axis of other long-chain saturated, mono-unsaturated and polyunsaturated fatty acids. These techniques could be applied to the study of brush border membrane (BBM) uptake of cholesterol along the CVA.

Variations in BBM fluidity along the CVA have been described in the Lewis rat (1) and in New Zealand white rabbit intestine (2). These studies have demonstrated that changes in membrane fluidity along the CVA are species specific; fluidity decreases as one advances along the CVA in the rabbit and increases with movement up the CVA in the Lewis rat. Yet, variation in glucose uptake along the CVA were similar in both species. These studies also demonstrated that compositional changes in the BBM occur as enterocytes advance along the CVA. It is controversial whether these alterations in BBM lipid composition and fluidity are responsible for the alterations in D-glucose uptake noted in these two studies.

There has been limited study of BBM fatty acyl and phospholipid composition in conditions of intestinal adaptation. Keelan et al (3) established that, in female Wistar rats, BBM phospholipid fatty acid composition was influenced by dietary fatty acid composition. In diabetic rats, they further established (4) that, not only did the dietary fat composition influence the structure of the BBM, but it also influenced glucose absorption in vesicles prepared from these rats. Unpublished observations have demonstrated concurrent but not necessarily congruous alterations in the lipid composition of the enterocyte microsomal membrane and the phospholipid metabolizing enzyme PEMP (phosphatidyl ethanolamine methyltransferase) and PCT (phosphotidylcholine transferase) as well as in the fatty acid desaturases of the microsomes and the BBM. It is unknown whether there are alterations in the enterocyte microsomal membrane lipid composition in enterocytes obtained from the villus fractions along the CVA. It is similarly unknown whether reported changes in BBM fluidity and lipid composition are associated with differences in the activity of the enterocyte BBM lipid transporting enzymes. Such studies can now be performed using the techniques which have been developed and reported in this thesis.

Studies are being considered to examine lipid uptake by the BBM along the CVA in diabetes mellitus. Enhanced uptake of glucose occurs acutely in the ileum but not jejunum of diabetic animals and chronically in both the jejunum and ileum. This increased glucose uptake may be due to "recruitment" of glucose carriers into enterocytes of the mid portion of the CVA (5). It is unknown whether small intestinal adaptation in diabetes mellitus involves alterations in lipid uptake along the CVA. This fractionation technique will be applied to the study of lipid uptake by BBMV isolated from animals with diabetes mellitus to determine whether changes in lipid uptake occur during small intestinal adaptation in diabetes mellitus.

Future studies may also include examining whether changes in BBM function in response to dietary modification affect all populations of enterocytes or only enterocytes at specific levels of the CVA. Uptake could be studied in animals of different ages to determine the effect of aging on uptake characteristics along the axis.

Thus, multiple avenues of study have been opened up to help improve our understanding the function of the small intestine. With improved knowledge of BBM composition and uptake of different lipid compounds in different situations, we may be better able to optimize nutrition in both health and disease.

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VITA

NAME: Robert Joel Fingerote

BIRTHPLACE: Winnipeg, Manitoba

BIRTHDATE: May 6, 1956

EDUCATION AND DEGREES

1974	Graduated from high school in Winnipeg
1976	University of Manitoba Faculty of Science Began studies, declared Major in Chemistry
1976	Admitted to University of Manitoba Faculty of Medicine
1977/78	During the summers, conducted research on the in vitro permeability of canine and human pericardium. On the basis of this research, was awarded Bachelor of Science in Medicine degree
1980	Graduated from University of Manitoba Faculty of Medicine
1980/81	Rotating internship, University of Toronto General Hospital
1981	Registered, Licentiate of the Medical Council of Canada
1981	Began General Surgery residency at the University of Manitoba
1983	Withdrew from the General Surgery residency program, February
1983	March, entered general medical practice, with clinical practice consisting of locum tenens family practice, geriatrics and emergency medicine
1984	Successfully completed Advanced Cardiac Life Support provider course June. Advanced Cardiac Life Support instructor course completed November
1985	Began residency training in Internal Medicine at the University of Calgary, July
1988	Successfully met the requirements of the American Board of Internal Medicine and designated a Diplomate certified in the specialty of Internal Medicine, September
1989	Completed Internal Medicine residency at the University of Calgary, June. Final 6 months of this program was spent as a Clinical Gastroenterology Fellow
1989	June. Issued specialty certification by the Royal College of Physicians and Surgeons of Canada in the specialty of Internal Medicine
1989	September. Admitted to Fellowship in the Royal College of Physicians and Surgeons of Canada

1989	Began research with financial support from Glaxo Pharmaceuticals in the Department of Internal Medicine, Division of Gastroenterology at the University of Alberta
1990	July. Began clinical gastroenterology Fellowship, University of Alberta
1991	Successfully met the requirements of the American Board of Internal Medicine and designated a Diplomate certified in the subspecialty of Gastroenterology, November

CURRENT MEMBERSHIPS

Alberta Medical Association
Canadian Association of Gastroenterology
American Gastroenterology Association
Registered on the Educational Registry of the Alberta College of Physicians and Surgeons

PRESENTATIONS

Poster

1988	Spontaneous mesencephalic hemorrhage Canadian Neurosciences Congress
1990	Aortoenteric fistula associated with acute myocardial infarction North American Conference of Gastroenterology Fellows
1991	Uptake of linoleic acid along the crypt-villus axis of rabbit intestine American Federation of Clinical Research

Podium

1991	Uptake of glucose but not linoleic acid is influenced by position of the enterocyte along the villus. North American Conference of Gastroenterology Fellows
1991	An approach to liver biopsy interpretation Workshop - Canadian Association of Pathologists with L.D. Jewell, M.D.

PUBLICATIONS

Papers

Fingerote, R.J., Shuaib, A., Brownell, A.K.W. Spontaneous nontraumatic midbrain hemorrhage. Southern Medical Journal 1990;83:280-282.

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Publications In Preparation:

Fingerote, R.J.,Thomson, A.B.R. Presence of a gradient for D-glucose but not linoleic acid uptake along the crypt-villus axis of rabbit jejunal.

Fingerote, R.J., Bain V.G. Fulminant Hepatic Failure.